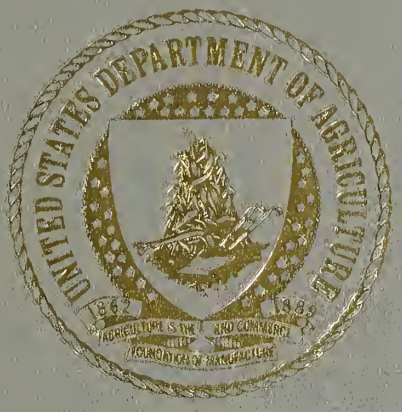
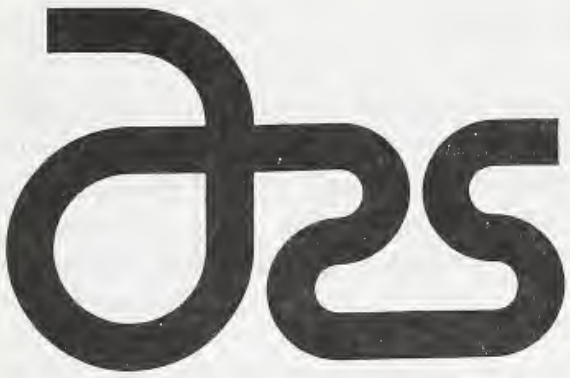
1998 PROGRESS REPORT ON FOOD SAFETY RESEARCH CONDUCTED BY ARS



Agricultural Research Service



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EXECUTIVE SUMMARY

This report summarizes ARS research progress on Food Safety in 1998. Included for the first time are ARS studies to control pathogens on fruits, vegetables and shellfish. The research is categorized into 5 general areas: **I. Control of Food borne Pathogens in Live Animals**; **II. Pathogen Control During Slaughter and Processing (Inspection Technology)**; **III. Post-slaughter Pathogen Modeling and Control**; **IV. Residue Detection and Chemical Analysis; and V. New Areas of ARS Research.**

I. Control of Food borne Pathogens in Live Animals:
Food safety of animal products begins with management practices, including herd and flock health programs to prevent disease and control infection in the live animal. ARS research to control human pathogens in live animals includes vaccine development, competitive exclusion cultures, and breeding and selection of resistant animals. This research benefits greatly from the long ARS experience with zoonotic pathogens in large animals.

ARS developed a competitive exclusion cultures (CEC) to control *Salmonella* on commercial broiler farms, and in March 1998, the FDA approved this CEC under the trade name PREEMPT™ for use in commercially produced broiler chickens. This was the first competitive exclusion culture to receive FDA approval for use in commercial poultry flocks. PREEMPT™ is a major milestone in an integrated program to prevent Salmonella contamination in food products from poultry.

Airborne transmission of *Salmonella* has gained considerable recognition as an important mechanism of spread of this pathogen through a group of birds. Electrostatic ionization of the air in an area housing *S. enteritidis*-infected adult birds reduced the number of *S. enteritidis* in the air environment. Airborne bacteria were also reduced in hatching cabinets with an electrostatic space charger. Methods to reduce Salmonella levels in the air become especially important when birds are highly susceptible, such as, shortly after hatch and during an induced molt.

As part of a National Antimicrobial Susceptibility Monitoring System in 1997, antibiograms were done on 2,391 *Salmonella* clinical and/or non-clinical isolates, including slaughter isolates. In 1998, the number of samples tested will double, and three additional sites have agreed to participate in supplying diagnostic clinical isolates, and the program has been expanded to include testing of *Campylobacter* and *E. coli* isolates. This monitoring program is conducted in collaboration with the FDA and the CDC and it will provide critical information to prolong the useful life of antibiotics for both human and animal use.

Research in several ARS laboratories seeks to enhance the host immune response to bacterial and parasitic infection, and thus decrease the dependence on antibiotic administration. A lymphokine was isolated from the splenic T cells of *S. enteritidis*-immune pigs. After oral administration, the lymphokine not only protected weaned pigs from S. *choleraesuis* organ invasion and cecal colonization, but also enhanced growth performance and neutrophil functions in the pigs. Separate studies with other swine cytokines indicate there are distinct periods of functional lymphocyte immaturity which may contribute to susceptibility to a variety of infectious agents. A porcine IL-12 has been produced that will be used to activate protective responses from neonatal pigs.

Optimized methods to identify, differentiate, and characterize pathogenic *E. coli* isolates from bovine sources were developed. Anti-O157 MAbs were also used in a blocking ELISA format to accurately detect serum antibodies to *E. coli* O157, as well as to the important non-O157 EHEC serotypes, *E. coli* O26 and *E. coli* O111, in cattle and other livestock. Serum detection of antibodies to *E. coli* 0157:H7 will allow accurate detection of all animals exposed to this pathogen, not just those that are shedding sufficient numbers at the time of fecal collection and examination.

Using laser technology, ARS scientists, in collaboration with University scientists, developed a detector that illuminates fecal contamination on meat. The instrument could immediately alert meat packers to contamination, allowing carcasses to be promptly decontaminated. A patent for the detector was filed in March, 1998.

II. Pathogen control during slaughter and processing (Inspection Technology):
Slaughter and processing are key links in the food safety chain. Improved understanding of food borne pathogen transmission, and control steps in prevention, sanitation, and processing technology are necessary elements to prevent contamination, cross contamination and wide spread food borne illness.

Construction and renovation of pilot plants that mimic slaughter and processing facilities are underway. At the Meat Animal Research Center the facility will have a special chamber with a belt-driven processing table to apply antimicrobials to beef trim meat. At the Richard Russell Research Center stunning and killing equipment in line with an electrical stimulator for poultry slaughter are being constructed. Physical and chemical interventions, sprays and dips, and other treatments will be tested in the simulated environments. Subsequent microbial analyses will measure the effects of the treatments. Optimum treatments will be determined, and new recommendations offered to aid the development of HACCP plans for commercial processors.

An instrument for monitoring chlorine dioxide during disinfection of food processing water has been developed, and a patent application filed. The membrane sensor determines chlorine dioxide in the presence of chlorine and/or other oxidants and gives instantaneous analytical results. The instrument can be used to assure both adequate residual levels and to minimize unnecessary overuse.

A bioluminescent strain of *E. coli* 0157:H7 was constructed having the same growth and attachment characteristics as the wild type strain. Bacterial attachment of this strain following artificial inoculation onto beef carcass tissues was monitored in real time by measuring light emission (bioluminescence). This research technology will aid in understanding the basis of microbial attachment and detachment to animal carcasses. Further, the technology offers a more rapid means to evaluate antimicrobial carcass treatments that do not rely on sampling, culturing and back-extrapolation of the resulting plate counts to large surface areas.

New methods are being devised to determine the efficacy of material treatments to render inanimate surfaces in the processing areas more resistant to bacterial contamination and biofilm formation. Physical and electrochemical treatments of stainless steel, including various methods of sanding,

grinding, and polishing, were tested for inhibition of bacterial attachment and biofilms. After treatment, each of the treated surfaces was less susceptible to bacterial attachment than untreated stainless steel. Stainless steel samples that had been electropolished showed significantly fewer bacterial cells and initial biofilm formations than all others tested. These findings will aid equipment manufacturers in selecting materials that are not conducive to pathogen growth.

A multiplex PCR method which simultaneously detects enterotoxigenic, attaching and effacing, and Shiga toxin-producing (both Stx1 and Stx2) *E. coli* strains from calves was developed in collaboration with Dr. H. Moon and S. Franck at Iowa State University (ISU). The multiplex PCR method is being used in diagnostic laboratories to identify, differentiate, and characterize pathogenic *E. coli* isolates from calves. The results will be useful to producer and veterinarians for the rapid diagnosis of all the diseases caused by *E. coli* in calves.

Mouse monoclonal antibodies (MAbs) that bind specifically to *C. jejuni* and *C. coli* have been developed, and the molecules they bind have been characterized. Use of these antibodies will improve the current detection methods for the *in situ* identification, biology, and attachment of *C. jejuni*. Rapid analysis of proteins in pathogenic strains of *Campylobacter* can be obtained by matrix-assisted laser desorption mass spectrometry, or MALDI. In the method, a single bacterial colony is analyzed. A double-blind analysis of 21 *C. jejuni* and *C. coli* correctly classified 20/21 strains based on comparison with hippuricase assay, PCR and MAb assignments. Mass spectrometric measurements have the potential to be widely used for confirmatory analysis of pathogenic bacteria.

Naturally occurring food additives in combination with other food chemicals were evaluated for their ability to inhibit attachment of bacterial pathogens to bovine fascia and connective tissues, and also to detach such pathogens from food surfaces. The inhibitors, for which a patent application will be filed, had varying abilities to block the binding of collagen-laminin with *E. coli* surfaces. Inhibition of *E. coli* O157:H7 attachment to intact meat tissues by use of these substances will offer an additional means to help to prevent *E. coli* contamination of food.

III. Post Slaughter Pathogen Modeling and Control:

Risk assessment is fundamental to evaluating the effect of production practices, processing and transportation systems on the contamination of food producing animals and plants. Rapid and accurate methods of detection, and quantitative measurement of pathogens at all critical points during food processing are needed to provide the necessary data to carry out risk assessment, to develop and validate predictive microbiological models, and to identify areas where interventions are most critically needed.

Green fluorescent protein (GFP)-expressing strains of *Salmonella* were used as a tool for modeling behavior of *Salmonella* in raw and cooked poultry products. The data were incorporated into the *Salmonella* - Risk Assessment Modeling Program for Poultry (*S*-RAMPP). Current simulation models in version 1.0 of *S*-RAMPP were made more user-friendly and Version 2.0 will be available in December 1998. A new simulation model, the Food Animal Risk Model for Poultry Pathogens (FARM-PP) will also be released in December 1998. FARM-PP predicts the severity of outcomes from consumption of poultry products contaminated with *Salmonella* and/or *Campylobacter*.

ARS in cooperation with IGEN of Gaithersburg, MD has developed an immunomagnetic electro-chemiluminescent (IM-EC) method for the detection of 1 cfu of *E. coli* O157:H7. The test is rapid, inexpensive, and user-friendly, requiring incubated for only 1 hour before analysis on an automated ORIGEN instrument. An antibody based determination of presumptive contamination by *E. coli* takes less than 1 minute. The technology is currently under industry evaluation, and preliminary data suggests the test will be 10-100 times more sensitive than those tests currently available.

Premature brown color in beef patties cooked at less than safe temperatures was known to present a potential food safety risk. To more completely delineate when color gives false information, ARS collaborated with FSIS laboratories to conduct a nationwide evaluation. The results showed that color in cooked patties changes very quickly and is often not equally distributed within a patty; in raw ground beef, considerable variation can exist between product surface and product interior and considerable variation in internal temperature can also exist within patties during cooking; and thawed ground beef produced more brown color when cooked than patties cooked fresh or rapidly thawed. Altogether the observations provided solid evidence that cooked beef patty color is not a good indicator of internal patty temperature. The results were a major factor in the development of the new FSIS consumer message that "consumers should not eat ground beef patties that are pink or red in the middle unless a food thermometer has been used to verify cooked temperature."

Glycine betaine, proline betaine, acetyl carnitine, carnitine and 3-dimethylsulfoniopropionate have been identified as osmoprotectants and cryoprotectants for *Listeria monocytogenes* as evidenced by an increase in growth rate of the pathogen during salt or chill-stress in defined media. The presence of osmoprotectants and cryoprotectants in foods is likely to help bacteria overcome the barriers of high osmotic strength and low temperature designed to control microbial growth. Identification of this type of compound will assist in identifying foods where added measures may be needed to control or eliminate the pathogen.

ARS scientists have evaluated the use of gamma irradiation for controlling the human pathogens *E. coli* 0157:H7 and *Salmonella* on seed used for the growth of sprouts and for the control of parasitic pathogens, such as, the coccidia *Cyclosporidium* and *Cryptosporidium*, on soft fruits such as berries. Initial results indicate that irradiation is efficacious and minimal levels destroy both bacterial and parasitic pathogens. This technology can significantly reduce pathogens in certain food commodities, while increasing shelf life and maintaining freshness, all major consumer demands.

IV. Residue Detection and Chemical Analysis:
Food safety research also includes the detection of residues of drugs, environmental toxins, natural toxins, and chemical analysis of nutrient quality.

ARS has a focused program on the development of methodology to detect drug and other chemical residues in eggs, an area which has heretofore received little attention. The methods developed include supercritical fluid extraction methods using carbon dioxide for the isolation of sulfonamides, chloramphenicol, and triazine herbicides, and microdialysis for isolation of fluoroquinolones such as sarafloxacin. Some of these drugs are prohibited from use in food producing animal, however there is still the potential for residues from illegal use. The methods will be transferred to FDA and to FSIS for their evaluation, and use, as dictated by their regulatory programs.

Valid and reliable laboratory methods for assaying iron were established as an indicator of soft bone constituents in trim beef derived form advanced meat recovery systems (AMRS). Studies indicated that iron content of AMR trim beef could be determined by either dry ash or wet ash (nitric/sulfuric) procedures, although the dry ash method was selected for routine analysis.

A method of analysis for multiple diverse pesticides was developed for fatty samples using extraction with acetonitrile, solid phase extraction clean-up and gas chromatography/ion trap mass spectrometric detection. The method is reasonably fast and easy, enables extraction of meat tissue as well as fat, expands the range of pesticides analyzed, uses no chlorinated solvents, and provides a single step quantitation and confirmation analysis. This method will increase the capabilities of regulatory and other laboratories to analyze pesticide residues in food, and will provide more accurate data for risk assessment purposes.

V. New Areas of ARS Research:
ARS scientists conduct research that is both basic and applied. Innovation is a key attribute, and new areas of study are constantly being undertaken.

Intact, cut and punctured apples, inoculated with non-toxigenic strains of *E. coli* O157:H7 were washed with various conventional and experimental wash formulations to determine their efficacy in decontaminating apples. Solutions containing 5% hydrogen peroxide, alone or in combination with acidic detergents and heated to 50C achieved 3-4 log reductions in intact and cut apples. Infiltration of *E. coli* 0157:H7 into apple core tissue was demonstrated when apples were immersed in a bacterial cell suspension, and the fruit temperature exceeded that of the water. These studies demonstrated that conventional methods of washing apples are largely ineffective and that even experimental methods cannot achieve a 5-log reduction.

E. coli develops extreme acid resistance when it is grown under mildly acidic conditions, and studies with *E. coli* O157:H7 indicated that undissociated volatile fatty acids could be an inducer. When cattle are fed large amounts of grain (> 45% of DM), volatile fatty acids accumulate in the colons causing a decline in pH. This causes a 1000-fold increase in total *E. coli* numbers, accompanied by a 1000-fold increase in *E. coli* acid resistance. Based on numbers and survival after acid shock, cattle fed grain had 1,000,000-fold more acid-resistant *E. coli* than cattle fed hay. When cattle were switched from grain to hay, total *E. coli* numbers, and acid-resistant *E. coli* decreased. After 5 days acid-resistant *E. coli* could no longer be detected. Although additional studies are required, it is possible that feeding hay to cattle prior to slaughter may significantly reduce post harvest contamination.

Conclusion:
ARS has an organized and productive research program on food safety of both animal and plant products. This research addresses prevention and control of pathogens, and residues at every aspect of the **farm to table continuum**; from prevention in the live animal or growing plant, to techniques of eliminating pathogens at the processing level, to storage, quality and the preparation of food products. The ARS research program in food safety is of vital importance to FSIS, and supports their efforts to continue to provide the consumer with the safest of food supplies.

1998 PROGRESS REPORT ON FOOD SAFETY RESEARCH CONDUCTED BY ARS

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Part I. CONTROL OF FOODBORNE PATHOGENS IN LIVE ANIMALS

CYTOKINE-MEDIATED MODULATION OF THE INNATE IMMUNE RESPONSE TO PREVENT SALMONELLOSIS IN POULTRY

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CRIS NUMBER: 6202-42000-008
FSIS CODE: C1MA02
CRIS TERMINATION DATE: April, 2000

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OBJECTIVE A: Develop a non-traditional immunological method to prevent and/or control salmonellosis in poultry.

PROGRESS A: During the past year, we demonstrated that the administration of *Salmonella enteritidis*-immune lymphokines (SILK) in day-old chickens and turkeys hasten the functional ontogeny of the primary granulocytic cell in poultry, the heterophil. During the first 7-14 days after hatch, the entire immune system is functionally immature with only limited capability of responding to pathogenic infections. The innate host defense system, featuring heterophils and macrophages as primary effector cells, control the numbers of organisms during initial infections. However, the cells of the innate system are also functionally immature during the first two weeks post-hatch. We have shown that following the administration of SILK, the functional activity of heterophils from 1-7 day-old birds is comparable to that of an immunologically mature bird. Mechanistically, these functionally mature heterophils are responsible for the protective inflammatory response, which protects the birds from salmonellae infections. Additionally, we have demonstrated over the last year that the prophylactic administration of SILK to day-old poultry reduces horizontal transmission of paratyphoid and typhoid *Salmonella* between birds. *In ovo* administration of SILK has also been shown to significantly reduce S. *typhimurium* colonization of the intestine. We have also demonstrated that the prophylactic administration of SILK will protect neonatal chickens against colibacillosis.

IMPACT/TECH TRANSFER A: A patent for the *in ovo* administration of SILK for the prevention of infectious diseases of poultry was issued this year. A second patent was issued for the production of the T-cell line to produce SILK. A CRADA with Eli Lilly and Company for the development and use of SILK for the control of poultry diseases continued until June 1998.

However, discussions have begun with Fort Dodge Animal Health for the formation of a CRADA for the development and use of SILK for the control of *Salmonella*, *E. coli*, and other poultry pathogens.

OBJECTIVE B: Optimize an effective delivery system(s) of SILK to neonatal poultry.

PROGRESS B: This year tests were performed to evaluate whether SILK could be administered to day-old chickens and turkeys by routes routinely used by the industry for vaccines and still be able to confer protection to the birds against localized enteric *Salmonella* infections. The results indicated that the delivery of SILK either orally, subcutaneously, or by aerosol confers significant protection against salmonellae infections that persists for up to six days. The mechanisms appear to be via SILK-mediated activation of heterophil function.

IMPACT/TECH TRANSFER B: These experiments suggest that SILK could be readily administered to poultry without new specialized equipment. We are working with Fort Dodge Animal Health and Cobb-Vantress Poultry Breeders to analyze the most cost-effective route to administer SILK for use by the poultry industry.

OBJECTIVE C: Identify and purify the effector cytokine(s) and clone the gene for mass production. Identify and clone genes for avian cytokines that might enhance host immune responses in neonatal poultry.

PROGRESS C: Initial studies have been conducted for the fractionization of SILK based on ammonium sulfate precipitation and the heat stable nature of the material. Monoclonal antibodies were generated against SILK and they were shown to neutralize various biological functions of SILK. Immune blots of material separated by gel electrophoreses demonstrated a protein approximately 30-40 kD in the SILK samples. Genes for interferon-gamma and the putative homologue of human IL-8, 9E3/CEF4 were cloned in both mammalian cells and *E. coli*. We have demonstrated that interferon-gamma will stimulate the functional activity of chicken heterophils isolated from day-old chickens.

IMPACT/TECH TRANSFER C: Purification of the SILK and/or isolation of the gene(s) of SILK and other avian cytokines will lead to the licensing of this technology.

OBJECTIVE D: Develop a *Salmonella*-immune lymphokine to control *Salmonella* infections in swine.

PROGRESS D: Using knowledge gained from the administration of *Salmonella enteritidis*-immune lymphokines (SILK) derived from the T cells of *S. enteritidis*-immune chickens to neonatal poultry, the idea of enhancing the immune response of immune-compromised animals was applied to swine. A lymphokine (PILK) was isolated from the splenic T cells of *S. enteritidis*-immune pigs. PILK was then administered orally to weaned pigs (considered to possess deficient immune responses) which were subsequently challenged with both lethal and non-lethal doses of the swine pathogen S. *choleraesuis* (SC). PILK-treated pigs were shown to have a 50-60% decrease in SC organ invasion and a similar reduction in cecal colonization. PILK also enhanced growth performance in both SC challenged and nonchallenged pigs, with PILK-treated pigs gaining an average of 5 pounds more than both nonchallenged controls and SC challenged controls. PILK also significantly reduced morbidity and mortality as compared to control pigs. Neutrophils isolated from the peripheral blood of PILK-treated pigs exhibited increased functional capabilities when compared to control pigs. Significant increases in the oxidative burst, adherence to nylon wool and bovine serum albumin-coated slides, and increased chemotaxis towards stimuli were shown by neutrophils from PILK-treated pigs when compared to neutrophils from control pigs. We have shown that PILK protects pigs from SC organ invasion, cecal colonization and enhances growth performance and neutrophil functions in weaned pigs.

IMPACT/TECH TRANSFER D: Discussions have begun with Fort Dodge Animal Health for the formation of a CRADA for the development and use of PILK for the control of *Salmonella choleraesuis, Actinobacillus pleuropneumoniae*, and other swine pathogens. The successful development of a PILK will provide swine producers a new technology to control salmonellosis and other infectious agents in swine.

OBJECTIVE E: Develop a *Salmonella*-immune lymphokine to control mastitis infections in dairy cattle.

PROGRESS E: A bovine immune lymphokine (BILK) was developed by immunizing a calf with *Salmonella enteritidis*. We then determined whether BILK would prevent the establishment of a *Staphylococcus aureus* mastitis in dairy cattle. A dose of BILK (50μg of protein) which produced no clinical response by itself was administered (into the teat) one milking before challenge. A combination of BILK and the antibiotic approved for mastitis control, Cefa-lak (50μg), was given to determine if any additional effects could be observed. Three groups: BILK only, Cefa-lak only, and BILK plus Cefa-Lak, were compared by assessing infected quarters beginning 4 h post challenge. Quarters refer to the teat quarters on a dairy cow (4 quarters/cow). BILK administered prior to *S. aureus* challenge prevented the establishment of mastitis in 12 quarters. Inclusion of Cef-lak did not contribute to the outcome. Combined, 24 quarters received BILK and were challenged with a dose of S. aureus known to produce a culture positive mastitis in 11 of 12 quarters that did not receive BILK. No *S. aureus* positive quarters were found in the BILK-treated animals

for up to 16 days post-challenge. In the Cefa-lak-treated animals, 11/12 quarters were *S. aureus* positive 1 day post-challenge. These quarters were retreated with Cefa-lak 2 days post-challenge. Five days later, 4 out of 12 quarters were still positive for *S. aureus*.

IMPACT/TECH TRANSFER E: The successful development of a BILK will provide dairy producers a new technology to control mastitis, *E. coli*, and other infectious agents in cattle ultimately reducing the need to use antibiotics.

PUBLICATIONS:

Bowden, L.L., V.K. Lowry, J.R. DeLoach, and M.H. Kogut. 1998. Age-dependent phagocytosis and bactericidal activities of the chicken heterophil. Develop. Comp. Immunol. 22:103-109.

Kogut, M.H., K.J. Genovese, R.B. Moyes, and L.H. Stanker. 1998. Evaluation of oral, subcutaneous, and nasal administration of *Salmonella enteritidis*-immune lymphokines on the potentiation of a protective heterophilic inflammatory response to *Salmonella enteritidis* in day-old chickens. Can. J. Vet. Res. 62:27-32.

Kogut, M.H., V.K. Lowry, R.B. Moyes, L.L. Bowden, R. Bowden, K. Genovese, and J.R. DeLoach. 1998. Lymphokine-augmented activation of avian heterophils. Poultry. Sci. 77:964-971.

Lowry, V.K., K.J. Genovese, L.L. Bowden, and M.H. Kogut. 1997. Ontogeny of the phagocytic and bactericidal activities of turkey heterophils and their potentiation by *Salmonella enteritidis*-immune lymphokines. FEMS Immunol. Med. Microbiol. 19:95-100.

Moyes, R. B., M. H. Kogut, R. E. Droleskey, and J. R. DeLoach. 1998. Differential expression of adhesion molecules by chicken heterophils activated *in vivo* with *Salmonella enteritidis* -immune lymphokines. Vet. Immunol. Immunopathol. 62:83-95.

PATENTS:

Method to Produce Granulocyte Colony Stimulating Factor From Immortalized Avian T Lymphocytes and Method to Produce Immortalized Cells. Patent Number 5,691,200. Issued November 25, 1997.

Method of Treating Birds. Patent Number 5,698,193. Issued December 16, 1997.

PATHOGENESIS, DETECTION, AND CONTROL OF *SALMONELLA ENTERITIDIS* AND OTHER SALMONELLAE IN CHICKENS

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CRIS NUMBER: 6612-32000-017
FSIS CODE: S1AM01
CRIS TERMINATION DATE: April, 2001

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OBJECTIVE A: Determine if (and how) the directed movement of air can lead to the transmission of *Salmonella enteritidis* infection between groups of chickens.

PROGRESS A: Direct contact between infected birds and exposure to contaminated environments are known to be potential sources of transmission of *S. enteritidis* in poultry, but the possibility of airborne dissemination of infection has received much less attention. In the present study, groups of chicks were housed at both ends of isolation cabinets in which air moved from an "upstream" area, across an unoccupied central area that prevented contact between chicks at either end, and into a "downstream" area. The upstream groups of chicks in each isolator unit were orally infected with *S. enteritidis*. When chicks from the downstream groups were removed and sampled several days later, *S. enteritidis* was found on the feathers of most chicks. Moreover, chicks in 50% of the downstream groups became infected with *S. enteritidis*. Because the surfaces of the downstream chicks were more often positive for *S. enteritidis* than were samples of tissues, and because samples of the intestinal tract were more often positive than were samples of lungs, it is likely that airborne movement of *S. enteritidis* caused environmental contamination in downstream units, after which oral ingestion by chicks led to internal infection.

IMPACT/TECH TRANSFER A: The results of this study suggest that reducing the airborne movement of *S. enteritidis* in poultry houses should help restrict the spread of infection within flocks. This information may accordingly provide the poultry industry with a tool for reducing the incidence of egg contamination by *S. enteritidis*.

OBJECTIVE B: Develop methods to reduce levels of *S. enteritidis* circulating in the air.

PROGRESS B: Laying hens undergoing an induced molt through feed removal are acutely susceptible to infection by *S. enteritidis*. Airborne *S. enteritidis* can serve as a source of infection for these birds during this period of high stress and methods were sought to reduce the numbers of *S. enteritidis* circulating in the air. Electrostatically ionizing an air environment causes airborne

particles to become negatively charged and they therefore become attracted to, and bind to, grounded surfaces such as walls and ceilings. Previous studies showed that this air ionization reduced the horizontal transmission of viral and bacterial pathogens between birds housed in a confined space. We examined whether charging the air within a large room area housing *S. enteritidis*-infected birds would, over time, affect the number of airborne organisms. In the first experiment a significant reduction, 85% or greater, was observed in counts on plates exposed for 24 hours in the room containing the ionizer compared with counts from the nonionizer room. However, the ionizer became less effective in reducing airborne *S. enteritidis* as time progressed, resulting from accumulation of dust and dander on the instrument. Cleaning the ionizer dramatically reduced the numbers of airborne *S. enteritidis*. In a repeat experiment, efficiency of plate count reduction by the ionizer exceeded 90%, compared with counts from the nonionizer room, if the instrument was routinely cleaned on alternate days. Further, while all of the plates exposed in the nonionizer room were culture positive by day 4 post challenge, 30%-80% of the plates from the ionizer room were entirely culture negative after day 6. The experiments were terminated after 10 days but it is expected that this reduction could continue for extended periods if instrument cleanliness was maintained.

IMPACT/TECH TRANSFER B: Airborne transmission of *Salmonella* has gained considerable recognition as an important mechanism of spread of this pathogen through a group of birds. This is especially true for individuals exposed during times of high susceptibility such as shortly after hatch and during an induced molt. Methods to reduce *Salmonella* levels in the air therefore become especially important at these times but also may have application at other stages in the life of the birds. Ionization of the air environment provides a relatively easy method to remove *Salmonella* and other poultry pathogens circulating in the air. Further, this technology also lowers dust levels in the house which would provide a significant health benefit to exposed employees. Use of this technology is still in its infancy, and it may have applications in other areas of food animal production.

OBJECTIVE C: Begin validating the reliability of using changes in LPS structure for hazard analysis of *S. enteritidis* in epidemiological studies.

PROGRESS C: An important attribute that contributes to the pandemicity and pathogenic potential of *S. enteritidis* is the ability of some strains to produce unusual types of lipopolysaccharide (LPS). Research conducted over several years correlates production of high-molecular-weight (HMW) LPS with enhanced parenteral invasiveness, high mortality in chicks and efficient egg contamination. Glucosylation of the HMW LPS structure appears to enhance oral invasiveness. Currently, there are some perplexing questions about these unusual LPS structures that can only be addressed by using genetic methods to confirm how HMW LPS and glucosylation of HMW LPS is regulated. To address these questions, a CSREES/NRICGP Ensuring Food Safety grant was written and awarded

to Dr. Guard-Petter in FY98. An experienced post-doctoral geneticist with appropriate experience in LPS biochemistry, Dr. Craig Parker, was hired and will begin work on the project early FY99.

IMPACT/TECH TRANSFER C: We propose that there are easy inexpensive assays that can be introduced into standard routines that will identify strains of *S. enteritidis* that are particularly hazardous to human health. The information about how these assays can be used will be transferred to FSIS scientists via meetings and published manuscripts. A book chapter describing using LPS structural analysis for differentiation of strains comes out in FY99. A review was published that raises some of the issues surrounding using LPS for strain differentiation. A medium for detecting strains of *S. enteritidis* that easily undergo swarm cell differentiation, which correlates with production of HMW LPS, was patented this year and is available to FSIS for licensure.

PUBLICATIONS:

Gast, R.K. 1997. Paratyphoid Infections, p. 97-121. *In:* B.W. Calnek (ed.) Diseases of Poultry. Iowa State University Press, Ames, Iowa.

Guard-Petter, J. 1998. Variants of smooth *Salmonella enterica* serovar *Enteritidis* that grow to higher cell density than the wild type are more virulent. Appl. Environ. Microbiol. 64:2166-2172.

Hogue, A., P. White, J. Guard-Petter, W. Schlosser, R. Gast, *et al* . 1997. Epidemiology and control of egg-associated *Salmonella enteritidis* in the USA. World Org. Animal Health Sci. Tech. Rev. (Rev. Sci. Tech. Off. Int. Epiz.) 16:542-553.

Holt, P.S., H.D. Stone, R.K. Gast, and R.E. Porter, Jr. 1997. Growth of *Salmonella enteritidis* (SE) in egg contents from hens vaccinated with an SE bacterin. Food Microbiol. 13:417-426.

Gast, R.K., and P.S. Holt. 1998. Supplementing pools of egg contents with concentrated enrichment media to improve rapid detection of *Salmonella enteritidis*. J. Food Protect. 61:107-109.

Gast, R.K., B.W. Mitchell, and P.S. Holt. 1998. Airborne transmission of *Salmonella enteritidis* infection between groups of chicks in controlled-environment isolation cabinets. Avian Dis. 42:315-320.

PATENTS:

Guard-Petter, J., and K. Ingram. 1998. A complex growth supplement for maintenance of bacterial cell viability and induction of bacterial cell differentiation. Patent approved, June 1998. Patent and Trademark Office, Serial Number 08/649,501.

CONTROL OF *SALMONELLA* IN DOMESTIC ANIMALS

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CRIS NUMBER: 6612-42000-019
FSIS CODE: R2AM02
CRIS TERMINATION DATE: September, 2000

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OBJECTIVE A: Develop on-farm control procedures to prevent colonization of chickens and/or turkeys with *Salmonella*.

PROGRESS A: Under a Cooperative Research and Development Agreement (CRADA) with the Continental Grain Company, our patented mucosal competitive exclusion (MCE) culture has been successfully scaled up for commercial production by CHR Hansen of Milwaukee, WI. All required submissions including manufacturing chemistry have been provided to FDA and final approval of the product is anticipated soon. In addition, Continental Grain has initiated commercial field trials in Brazil and Japan. Investigations of application of the yeast, *Saccharomyces boulardii*, to poultry feed to reduce *Salmonella* colonization continue. We have assisted our CRADA partner in setting up large scale pen and field trials where more than 200,000 broilers will be tested with the yeast diets. We have also shown in floor pen trials that litter treatments can successfully reduce *Salmonella* colonization in chickens grown on contaminated litter.

IMPACT/TECH TRANSFER A: Competitive exclusion, yeast and litter treatments each offers the possibility of being able to significantly reduce the on-farm colonization of chickens with *Salmonella* thus helping the poultry industry to meet specified *Salmonella* standards.

OBJECTIVE B: Develop and test technology to lower particulate dust and associated airborne bacteria in the hatching cabinet.

PROGRESS B: In cooperation with Dr. Bailey Mitchell, engineer at the Southeast Poultry Research Laboratory an electrostatic space charger was placed into a hatching cabinet. Studies were carried out showing the efficacy of the space charger to lower the amount of particulate dust, total aerobic bacteria, Enterobacteriaceae and *Salmonella* in the air of a hatching cabinet during a broiler chicken hatch. The effect was also studied by growing chicks for one week and noting a decreased level of intestinal colonization with *Salmonella*.

IMPACT/TECH TRANSFER B: This technology is currently under investigation in the commercial hatchery industry as a viable means to increase hatchery sanitation and reduce the impact of microbial cross-contamination during the hatching process.

OBJECTIVE C: Determine changes in the intestinal microbiology of commercial turkeys in response to administration of coccidiostats and growth promoters.

PROGRESS C: Under a CRADA with a major pharmaceutical company, a study was conducted with a commercial breed of turkey hens and feed formulations found in the turkey industry. Changes in populations of some groups of bacteria were noted under certain feed additive combinations, i.e., drops in numbers of clostridia and lactobacilli. However, the changes occurred in only certain portions of the intestine and were transient. The results showed no clear-cut relationship between feed additives and presence of *Salmonella* and *Campylobacter* in turkey cecae.

IMPACT/TECH TRANSFER C: Use of antibiotics is known to result in increases and decreases of specific types of intestinal microbes including animal pathogens and human pathogens transmitted through animal foods.

OBJECTIVE D: Determine the sources of *Clostridium perfringens* in poultry production and develop epidemiological risk assessment models.

PROGRESS D: A preliminary study was conducted to determine the most effective methodology for sampling wild bird populations as potential sources of salmonellae, *Campylobacter jejuni*, and *Clostridium perfringens* and the most effective methodology for isolating *C. perfringens* from poultry production and processing samples. In samples collected from 16 farms from 4 poultry integrators and from the associated processing plants , over 1,000 strains of *C. perfringens* have been isolated. Some of these positive samples are from sources not previously reported in the scientific literature.

IMPACT/TECH TRANSFER D: Little information exists about the incidence or sources of *C. perfringens* in poultry production. Epidemiological data on sources and spread of this poultry/human pathogen in poultry production is needed in order to develop effective risk assessment models for these pathogens.

OBJECTIVE E: Test the efficacy of chemical immersion sanitization of hatching eggs to lower the incidence of *Salmonella* contamination.

PROGRESS E: Two studies were completed in the area of egg immersion. One set of experiments was done to test the use of multiple immersions with and without a surfactant. A second set of experiments were done to test application of hydrogen peroxide with a vacuum and a surfactant. This treatment proved to be effective to control *Salmonella* contamination without affecting hatchability.

IMPACT/TECH TRANSFER E: Previous research has shown that the hatchery is the most critical point for control of *Salmonella* during broiler production. These studies add to and enhance our previous studies showing the possibilities of chemically disinfecting fertile hatching eggs to prevent cross-contamination and spread of *Salmonella* in the hatchery and on the farm.

OBJECTIVE F: Compare plating media to determine efficacy in the recovery of *Salmonella* from poultry products.

PROGRESS F: Seven plating media were compared for ability to detect *Salmonella* from chicken carcass rinse samples. The current method of BGS and MLIA combined proved to very effective. Variation was seen among the other media tested.

IMPACT/TECH TRANSFER F: The FSIS method was validated and much of the variation seen in lab to lab comparisons was explained due to media differences.

OBJECTIVE G: Determine the extent of salmonellae contamination of turkeys in commercial production.

PROGRESS G: Four commercial turkey flocks were examined for presence of salmonellae by examination of droppings at ages of 6, 10 and 15 weeks. Salmonellae contamination was noted in all flocks at levels ranging from 10 to 40 % throughout production.

IMPACT/TECH TRANSFER G: Salmonellae contamination in turkeys occurred at different ages and levels than in broilers indicating that intervention strategies may need to be developed specifically for turkeys.

OBJECTIVE H: Determine an effective and safe means to sanitize ostrich eggs to lower the presence of the ostrich pathogen, *Salmonella.*

PROGRESS H: Three methods of ostrich egg de-contamination were tested and compared. Formaldehyde fumigation, a traditional hatchery method, was found to be more effective than a chlorine spray wash. However, still a low number of *Salmonella* can avoid the treatment leading to

potential cross contamination in the hatching cabinet. Application of hydrogen peroxide with a vacuum to go beneath the shell showed promise as a means to deliver the chemical to the *Salmonella* cells below the surface.

IMPACT/TECH/TRANSFER H: While most *Salmonella* do not harm chickens, *Salmonella* can be deadly to the ostrich chick. A reliable means to eliminate this microorganism from the shell and membrane prior to incubation is important to the industry.

PUBLICATIONS:

Bailey, J.S. 1998. Detection of *Salmonella* cells within 24 to 26 hours in poultry samples with the polymerase chain reaction BAX system. J. Food Protect. 61: 792-795.

Berrang, M.E., J.F. Frank, R.J. Buhr, J.S. Bailey, and N.A. Cox. 1998. Eggshell characteristics and penetration by *Salmonella* through the productive life of a broiler breeder flock. Poultry Sci. (in press)

Berrang, M.E., J.F. Frank, R.J. Buhr, J.S. Bailey, N.A. Cox, and J.M. Mauldin. 1997. Microbiology of sanitized broiler hatching eggs through the egg production period. J. Appl. Res. 6:298-305.

Chen, M., N. J. Stern, J. S. Bailey, and N. A. Cox. 1998. Optimum method to treat broiler chicks with mucosal competitive exclusion flora for control of salmonellae. J. Appl. Poultry Res. (in press)

Cox, N.A., J.S. Bailey, M.E. Berrang, and J.M. Mauldin. 1997. Diminishing incidence and level of salmonellae in commercial broiler hatcheries. J. Appl. Poultry Res. 6:90-93.

ABSTRACTS:

Bailey, J. S., J. E. Line and N. A. Cox. 1998. Comparison of internal colonization to external carriage of *Salmonella* on broiler chickens. Poultry Sci. 77 (suppl. 1):115.

Bailey, J.S., N.A. Cox, and N.J. Stern. 1998. Commercial field trial evaluation of Mucosal Starter Culture ™ to reduce *Salmonella* during broiler production. Poultry Sci. 77 (suppl. 1):115.

Bailey, J.S., N.A. Cox, and N.J. Stern. 1998. Ecology and control strategies for salmonellae in broiler chickens. Annu. IAMFES Conf. 66.

Bailey, J.S. 1998. The role of microbiological testing in HACCP. Proc. Int. Symp. Food-borne *Salmonella* in Poultry, Baltimore, MD, 35.

Bailey, J.S., N.J. Stern, and N.A. Cox. 1998. Mucosal starter culture™ treatment significantly reduces salmonellae on processed broiler carcasses. Proc. 6th Asian Pacific Poultry Conf. Nagoya, Japan, 442-443.

Berrang, M.E., N.A. Cox, R.J. Buhr and G.N. Rowland. 1998. Application of vacuum and hydrogen peroxide to eliminate *Salmonella* on ostrich eggs. Poultry Sci. 77 (suppl. 1):114.

Berrang, M.E., N.A. Cox, S.E. Craven, and N.J. Stern. 1998. Effect of sub-therapeutic levels of antibiotics in feed on the intestinal carriage of *Campylobacter* and *Salmonella* in turkeys. Poultry Sci. 77 (suppl. 1):96.

Cox, N.A., N.J. Stern, J.S. Bailey and M.E. Berrang. 1998. Prevalence of salmonellae in the cecal droppings of turkeys during production. Poultry Sci. 77 (suppl. 1):114.

Cox, N.A., M.E. Berrang and R.J. Buhr. 1998. Hydrogen peroxide applied with vacuum and a surfactant to eliminate *Salmonella* from hatching eggs. Poultry Sci. 77 (suppl. 1):20.

Cox, N.A., M.E. Berrang and J.S. Bailey. 1998. Efficacy of multiple dips to eliminate *Salmonella* from hatching eggs. Poultry Sci. 77 (suppl. 1):21.

Cox, N.A., and M.E. Berrang. 1998. Comparison of seven different selective plating media with naturally occurring *Salmonella* from processed broiler carcasses. 1998. Poultry Sci. 77 (suppl. 1):149.

Craven, S.E., T.S. Cummings, N.A. Cox, and N.J. Stern. 1998. Changes in the intestinal bacteria of turkeys after removal of the anticoccidial, monensin, and addition of growth-promoting antibiotics to the diet. Poultry Sci. 77 (suppl. 1): 24.

Mitchell, B.W., R.J. Buhr, M.E. Berrang, N.A. Cox and J.S. Bailey. 1998. Characterization and reduction of airborne articulates in the hatching cabinet with an electrostatic space charge. Poultry Sci. 77 (suppl. 1):146.

Mitchell, B.W., R.J. Buhr, M.E. Berrang, J.S. Bailey and N.A. Cox. 1998. Reduction of airborne bacteria in the hatching cabinet with an electrostatic space charger. Poultry Sci.77 (suppl. 1):151.

Stern, N.J., J.S. Bailey, and N.A. Cox. 1998. Mucosal starter culture™ as part of an integrated program for salmonellae control on processed carcasses. Proc. 10th Europ. Poultry Conf. Jerusalem, Israel, vol II:709-713.

Stern, N.J., J.S. Bailey and N.A. Cox. July 28, 1998. Mucosal Starter Culture™ (MSC™) Microflora for the Reduction of *Salmonella* in Poultry. FSIS Conf. Technology to Improve Food Safety. Washington, D.C.

PATENTS:

Line, E.L., N.J. Stern, N.A. Cox, and J.S. Bailey. *Ovo* antibiotic and microbial treatment to diminish salmonellae populations in avians. Issued March 3, 1998, U.S. Patent # 5,722,342.

EPIDEMIOLOGY AND CONTROL OF *SALMONELLA* IN SWINE

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FSIS CODE: N1AM01
CRIS TERMINATION DATE: June, 2002

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OBJECTIVE A: Determine the epidemiologic factors that may be subjected to intervention measures for control of *Salmonella* in swine.

PROGRESS A: A 31-pig study was conducted to investigate the effect(s) of stress on porcine salmonellosis. Pigs were infected with 10^6 *Salmonella choleraesuis* and held until they no longer shed detectable *Salmonella* in their feces. The animals were then treated with 2-deoxy-D-glucose (2DG) and dexamethasone to induce stress. Preliminary results using this experimental stress model, developed in our laboratory, indicate that stress does not necessarily cause recrudescence of *Salmonella* shedding even when tissues are culture positive for *Salmonella*. A new paradigm may be needed to accurately describe the *Salmonella* carrier-state in swine. That is, carriers should be divided into two subgroups, a larger group consisting of carriers unaffected by stress, and a smaller group consisting of persistent shedders which cycle between the active and inactive carrier-state.

IMPACT/TECH TRANSFER A: Results from this study further define the role of stress in porcine salmonellosis using a reproducible experimental model. The smaller group which is affected by stress could be an important source in foodborne *Salmonella* outbreaks. This work is an important first step for researchers interested in developing intervention strategies associated with pathogens such as *Salmonella*. Results have been presented to commodity groups and other professionals at scientific meetings.

OBJECTIVE B: Define the immune response associated with acute and chronic *Salmonella* infection of swine.

PROGRESS B: Field data suggests that recovery of *Salmonella* from finisher pigs may be variable and that only a small percentage of animals are long-term carriers. It is possible that certain swine are resistant to salmonellosis. Genetic control of disease resistance by selective breeding programs has become a viable proposition for disease control. We have developed an *in vitro* porcine macrophage bactericidal assay using peripheral blood and flow cytometry. In conjunction with a commercial breeder, we have screened a large number of pigs and successfully separated animals

into *Salmonella* "susceptible" and "resistant" pigs based on their ability to kill *Salmonella* in our *in vitro* macrophage bactericidal assay. Together with collaborators at Iowa State University we have begun to compare ability to kill bacteria with genotypic differences at genes relevant to the innate resistance pathway. Validation of the *in vitro* bactericidal assay with an *in vivo* challenge experiment is in progress.

Monoclonal antibodies to porcine tumor necrosis factor receptor-1 (TNFR-1) have been further characterized and evaluated as potential reagents for a highly sensitive and specific ELISA test. A better understanding of the porcine TNFR-1 and TNF response to salmonellosis may provide an avenue for therapeutic intervention.

IMPACT/TECH TRANSFER B: By studying porcine mechanisms of defense against *Salmonella* at the molecular level it will be possible to discover new therapeutic avenues for intervention against salmonellosis and other diseases. Levels of TNFR-1 in the blood may be of diagnostic or prognostic significance in salmonellosis. A blood-based assay to initially separate pigs into two groups, resistant and susceptible, will allow more focused screening of genetically defined breeding stock for altered genes that may predispose them to *Salmonella* or other intracellular pathogens. The consumer, breeders, producers, and scientists in related areas will benefit from the information and products generated from this research. The development of *Salmonella*-resistant swine would not only reduce the need for antibiotics and vaccines but lower the incidence of *Salmonella* in pork.

PUBLICATIONS:

Bosworth, B.T., and T.J. Stabel. 1998. Alimentary disease and bacteria after weaning. Proc. 15th Int. Pig Vet. Soc. Congr., Birmingham, UK. pp. 63-70.

Maroushek-Boury, N., B.T. Bosworth, T.J. Stabel, M.E. Kehrli Jr., and M.J. Taylor. 1998. Cloning and expression of porcine recombinant, soluble tumor necrosis factor receptor 1. Am. J. Vet. Res. (in press)

Stabel, T.J. 1997. Stress and porcine immunity to *Salmonella*. Proc. Allen D. Leman Swine Conf. pp. S8-S13.

Stabel, T.J. 1998. Evaluation of 2-deoxy-D-glucose as a stress inducer in swine. Am. J. Vet. Res. (in press)

NATIONWIDE EPIDEMIOLOGY SURVEY OF *SALMONELLA* AND *CAMPYLOBACTER* DURING BROILER PRODUCTION

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CRIS NUMBERS: 6612-42000-019/23
FSIS CODES: R2AM01, R2AM02
CRIS TERMINATION DATE: September, 2000/May 2002

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OBJECTIVE A: To identify and quantify sources of *Campylobacter* and *Salmonella* through all phases of broiler production.

PROGRESS A: Prior to beginning the nationwide epidemiology project, a pilot study was conducted on a poultry house in Georgia. *Salmonella, Campylobacter, and Clostridium perfringens* were isolated from fecal and environmental sources. Culture techniques were optimized, and genetic typing methodologies were compared. In early 1998, the nationwide project was initiated. Both high and low production farms from Georgia, Alabama, Arkansas, and California are being sampled seasonally (Spring, Summer, Fall and Winter) for one year. Samples are being taken at chick placement through broiler transport and processing at 2 week intervals, including both fecal and environmental samples. Genetic typing tools will be employed for traceback identification from the processed carcass to an initial source of contamination. Antibiograms, serotyping and phage typing will be included where appropriate to further characterize the isolates. Over 6,000 samples have been processed, and the sampling is approximately 50% complete. FSIS has assisted in this project by providing a $300,000 grant. Project completion is expected by December, 1999.

IMPACT/TECH TRANSFER A: This project will facilitate transfer of information to regulatory agencies, research institutions and the poultry industry who will then be able to focus on intervention strategies which will have the highest probability of reducing pathogen load.

OBJECTIVE B: To define and target effective intervention strategies and evaluate their results

PROGRESS B: As part of the nationwide epidemiology project, a risk factor assessment is being conducted which will result in identification of areas in which implementation of intervention strategies will have the highest probability of success. A risk-factor questionnaire has been developed and data is being gathered at every sampling time period. Following analysis in late 1999,

intervention strategies will be implemented on 2 to 4 houses which will have the highest likelihood of demonstrating differences pre- and post-intervention.

IMPACT/TECH TRANSFER B: This project will facilitate transfer of information to regulatory agencies, research institutions and the poultry industry. Implementation of successful strategies will result in a decreased pathogen load in final product.

ABSTRACTS:

Bahnson, P.B., and P.J. Cray. 1998. The association between herd characteristics and *Salmonella* in slaughter age pigs. Proc. Int. Symp. Epi. Cont. *Salmonella* Pork.

Bahnson, P.B., and P.J. Cray. 1998. *Salmonella* on farms: production factors associated with high prevalence. Proc. Allen D. Leman Swine Conf.

Baum, D.H., D.L. Harris, B. Nielsen and P.J. Cray. 1998. Epidemiologic studies of *Salmonella* on epidemiology and control of *Salmonella* in pork. Proc. Int. Symp. Epi. Cont. *Salmonella* Pork.

Baum, D.H., D.L. Harris, B. Nielsen and P.J. Cray. 1998. Risk factors associated with increased seroprevalence of *Salmonella* in finishing swine. Proc. Int. Symp. Epi. Cont. *Salmonella* Pork.

Craven, S.E., E. Line, N.J. Stern, J.S. Bailey, N.A. Cox, R.J. Meinersmann and P.J. Cray. 1998. Incidence of bacterial enteropathogens in wild-bird samples collected from a farm housing broiler chickens. Poultry Sci. Assoc. Meet.

Cray, P.J., J.D. McKean and G.W. Beran. 1998. Prevalence of *Salmonella* in swine and pork: a farm to consumer study. Proc. Food Safety Consortium.

Cray, P.J., J.D. McKean and G.W. Beran. 1998. Tracking *Salmonella* on the farm: a farrow to finish study. Proc. Int. Symp. Epi. Cont. *Salmonella* Pork.

Cray, P.J. 1998. Mechanism of host-agent interactions in subclinical *Salmonella* infection in pig herds. Proc. Int. Symp. Epi. Cont. *Salmonella* Pork.

Cray, P.J., R.W. Wills, T.J. Stabel, YK. Yoon, J.T. Gray and S. Zimmerman. 1998. Co-infection with *Salmonella choleraesuis* and porcine reproductive and respiratory syndrome (PRRS) virus. Proc. Int. Symp. Epi. Control *Salmonella* Pork.

Cray, P.J., J.S. Bailey, N.J. Stern and N.A. Cox. 1998. Mucosal competitive exclusion on epidemiology and control of *Salmonella* in pork. Proc. Int. Symp. Epi. Control *Salmonella* Pork.

Cray, P.J., N.A. Buchmeier, S. Bossie, D.G. Guiney, F. Fang, T. Testerman and S.J. Libby. 1998. Analysis of defined *Salmonella choleraesuis* mutants in swine. Res. Work. Anim. Dis. Conf..

Cray, P.J., N.J. Stern, J.S. Bailey, S.E. Craven and N.A. Cox. 1998. Recovery of *Salmonella, Campylobacter jejuni* and *Clostridium perfringens* from a poultry broiler house. Annu. IAMFES Meet.

Wells, S.J., P.J. Cray and E.K. Kabagambe. 1998. Prevalence and risk factors for *Salmonella* shedding on U.S. dairy operations. Am. Dairy Sci. Assoc.

NATIONAL ANTIMICROBIAL SUSCEPTIBILITY MONITORING

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OBJECTIVE: Monitor *Salmonella* and *Campylobacter* isolates from the FDA/CDC national Antimicrobial Susceptibility Monitoring System

PROGRESS: Testing for the veterinary arm of the National Antimicrobial Resistance Monitoring Program (NARMS) is conducted in this laboratory. The NARMS is an interagency endeavor involving the USDA-ARS, USDA-APHIS, USDA-FSIS, FDA-CVM, and CDC. It is one of very few projects involving the number of agencies it does. In 1997, antibiograms were done on 2,391 *Salmonella* isolates from clinical and non-clinical isolates including slaughter isolates. In 1998, the number of samples tested will double. Additionally, 3 sentinel test sites have agreed to participate in supplying diagnostic clinical isolates. The program has expanded to include testing of *Campylobacter* and *E. coli* isolates.

IMPACT/TECH TRANSFER: This project will facilitate transfer of information to regulatory agencies, research institutions, commodity groups, pharmaceutical industries and the veterinary and medical community. Analysis of this information will facilitate prudent and judicious use of antimicrobics.

ABSTRACTS:

Cray, P.J., D.A. Dargatz, M. Miller and L. Tollefson. 1998. Antimicrobial susceptibility patterns of *Salmonella* isolates of veterinary origin. Proc. AAVPT.

Cray, P.J., L. Tollefson and F.J. Angulo. 1998. National and international antimicrobial resistance monitoring efforts: a description of the U.S. monitoring systems - the national antimicrobial susceptibility monitoring system. Proc. Pig Vet. Soc. Int. Cong.

Cray, P.J., L. Tollefson and F.J. Angulo. 1998. National and international antimicrobial resistance monitoring efforts: a description of the U.S. monitoring systems - the national antimicrobial susceptibility monitoring systems. Proc. World Cong. Food Hyg.

Cray, P.J., M.A. Miller, D.A. Dargatz and L. Tollefson. 1998. Antimicrobial resistance patterns of *Salmonella* isolates of veterinary origin. Poul. Sci. Mtg.

Cray, P.J., H.L. Holcomb, D.A. Dargatz, M.A. Miller and L. Tollefson. 1998. Antimicrobial susceptibility patterns of *Salmonella* isolates of veterinary origin. Proc. Pig Vet. Soc. Int. Cong.

Cray, P.J., H.L. Holcomb, E.J. Bush, D.A. Dargatz and L. Tollefson. 1998. Resistance and sensitivity patterns of *Salmonella* isolates. Allen D. Leman Swine Conf.

Cray, P.J., M.A. Miller, D.A. Dargatz and L. Tollefson. 1998. Antimicrobial susceptibility monitoring. Am. Vet. Med. Assoc.

Cray, P.J., M. Miller, L. Tollefson and D.A. Dargatz. 1998. Antimicrobial susceptibility patterns for *Salmonella* isolates of veterinary origin for 1997. Annu. Amer. Soc. Microbiol.

Cray, P.J., D.A. Dargatz, M.A. Miller and L. Tollefson. 1998. Veterinary perspective on the use of antibiotics in animal production systems. Life Sci. Inst. Int.

Dargatz, D.A., P.J. Cray and N.E. Wineland. 1998. Description of the U.S. monitoring systems - the national antimicrobial susceptibility monitoring system. Proc. Swine Summit.

Holcomb, H.L., P.J. Cray, E.J. Bush, D.A. Dargatz and L. Tollefson. 1998. Antimicrobial susceptibility of epidemiology and control of *Salmonella* in pork. Proc. Int. Symp. Epi.. Cont. *Salmonella* Pork.

Miller, M.A., P.J. Cray, V. Toelle, K. Hollinger and L. Tollefson. 1998. Multiple resistance patterns of *Salmonella* isolates of veterinary origin. Emerg. Infect. Dis.

Miller, M.A., P.J. Cray and L. Tollefson. 1998. Antibiotic resistant *Salmonella* strains: characteristics and consequences. Intersci. Conf. Antimicrobial Agents Chemother.

Miller, M.A., P.J. Cray and L. Tollefson. 1998. Antibiotic resistant *Salmonella* strains: characteristics and consequences. Amer. Dairy Sci. Assoc..

Tollefson, L., M. Miller, P.J. Cray, F. Angulo and S. Sundlof. 1998. FDA/CDC/USDA antibiotic resistance monitoring program. J. Poultry. Sci.

DEVELOPMENT OF COST EFFECTIVE MEANS TO PREVENT AND CONTROL SALMONELLOSIS IN POULTRY

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CRIS NUMBER: 6202-42000-011
FSIS CODE: C1AM01
CRIS TERMINATION DATE: September, 2002

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OBJECTIVE A: Develop effective means to prevent/control *Salmonella* colonization of broiler chickens during growout to market age.

PROGRESS A: Final FDA pivotal field studies to evaluate the efficacy of patented competitive exclusion culture (CE) in commercial broiler flock were completed. Our CRADA partner, BioScience Division of Milk Specialties Company, Dundee, IL, completed the final report and submitted it for FDA approval and registration.

IMPACT/TECH TRANSFER A: In March 1998, the FDA approved the use of Milk Specialties' produced CF3 culture under the tradename PREEMPT™ for use in commercially produced broiler chickens. This was the first competitive exclusion culture to receive FDA approval for use in commercial poultry flocks.

OBJECTIVE B: Evaluation of a CE product, PREEMPT™ to prevent colonization of chickens by other pathogenic bacteria.

PROGRESS B: Newly hatched chicks have a microbiologically immature gastrointestinal tract and are easily infected with pathogenic bacteria. One approach to prevent the colonization of pathogenic bacteria is to establish a normal intestinal flora in chicks as early as possible to help combat these pathogens. We recently demonstrated that a CE product can reduce the colonization of newly hatched chicks by *Salmonella gallinarum*, *Clostridium perfringens*, *Listeria monocytogenes*, and the antibiotic-resistant *Salmonella typhimurium* DT104.

IMPACT/TECH TRANSFER B: The demonstration that competitive exclusion cultures are effective in reducing the gastrointestinal colonization by antibiotic resistant bacteria or other pathogenic bacteria indicates the potential of CE products as a pre-process pathogen intervention strategy.

OBJECTIVE C: Evaluate the chicken crop as a source of *Salmonella* and *Campylobacter* contamination of poultry slaughter plants.

PROGRESS C: Feed is withdrawn from broiler chickens 6 to 12 hours before transport to the processing plant. During feed withdrawal, birds peck at fecal droppings and litter on the rearing house floor that may contain pathogenic bacteria. Our laboratory recently demonstrated that both *Salmonella* and *Campylobacter* contamination of the crop increases dramatically during this feed withdrawal period. Additionally, we found that feed withdrawal resulted in a decrease in lactic acid concentration in the crop, accompanied by an increase in crop pH, which may account for the increase in crop contamination. These results strongly suggest that preslaughter feed withdrawal is a critical control point for reducing potential *Salmonella* and *Campylobacter* contamination of processed poultry meat products.

IMPACT/TECH TRANSFER C: Preliminary data from our laboratory suggest that specific acids provided to broilers during an 8 hour feed withdrawal will maintain crop pH and will provide an unfavorable environment for pathogenic bacteria. Presently, talks are underway with 2 large broiler production companies in Texas and Georgia to use this cost-efficient strategy to reduce *Salmonella* and *Campylobacter* contamination on commercial broiler farms.

OBJECTIVE D: Evaluate the effect of feed withdrawal during forced molt on *Salmonella* survival in the crops of Leghorn hens and investigate the mechanism(s) by which feed withdrawal during forced molt reduces resistance to *Salmonella*.

PROGRESS D: Forced molt induced by feed withdrawal is used to stimulate egg laying in aging flocks of hens. Feed removal during forced molt decreases the resistance of hens to *S. enteritidis* resulting in severe infections and organ invasion. The mechanism by which feed withdrawal decreases resistance to infection is unknown. We recently reported that preslaughter feed withdrawal in market age broilers results in increased *Salmonella* crop contamination and that crop contamination is directly associated with decreased concentrations of crop lactic acid and increased crop pH. Similarly, we found that during forced molt, increased *S. enteritidis* crop contamination is also associated with a decrease in crop lactic acid and an increase in pH. The results suggest that a common mechanism may account for the increased incidence of crop contamination during preslaughter feed withdrawal in broiler flocks and forced-molted aging hens.

IMPACT/TECH TRANSFER D: Increased survival of *Salmonella* in the crops of broilers during preslaughter field withdrawal and Leghorn hens during forced molt is clearly associated with decreased acidity of the crop. Provision of acids in the drinking water of broilers and layer hens during periods of feed withdrawal reestablishes the acidity of the crop and reduces *Salmonella* crop contamination.

OBJECTIVE E: To identify and characterize factors effecting attachment and colonization of the chicken gastrointestinal tract by *Campylobacter*.

PROGRESS E: We established methods for studying colonization of chicks by *Campylobacter jejuni*, and entered into a Cooperative Research Agreement with Washington State University. This cooperation has permitted us to study the effects of specific mutations in *Campylobacter jejuni* on the ability of *Campylobacter* to colonize the gastrointestinal tract of chickens. Two gene products have been identified which appear to play an essential role in colonization of the intestinal tract. These genes are dnaJ, which regulates production of a bacterial environmental stress response protein, and cad F, which codes for a protein constituent of the bacterial outer membrane. Additional *C. jejuni* strains with known genetic defects are also being studied. The cad F-deficient strain is especially interesting, since it is possible to produce the gene product in sufficient quantity that it can be tested as a possible immunogen.

IMPACT/TRANSFER E: Knowledge at the molecular level concerning bacterial attachment and intestinal colonization should strengthen the overall understanding of natural processes controlling enteric pathogens. Our research finds have been prepared for publication in the scientific literature. One manuscript is in press and one has been submitted.

OBJECTIVE F: Evaluate the effects of specific dietary constituents and toxins on the efficacy of indigenous flora and Competitive Exclusion (CE) Cultures to protect against salmonellae colonization in broiler chicks.

PROGRESS F: Disruption of the native microflora or administered CE cultures by dietary components or additives could affect the concentrations of volatile fatty acids (VFA), perhaps making the chickens more susceptible to colonization by *Salmonella* or and other enteropathogens. Studies were conducted using broiler chicks fed unmedicated diets (controls) or these unmedicated diets containing added aflatoxins, T-2 toxin, or tannic acid. Feeding relatively high concentrations of any of these additions caused a decrease in cecal propionic acid and/or total VFA. When a high concentration of T-2 toxin was fed to chicks administered a CE culture, they were more susceptible to cecal colonization by *Salmonella typhimurium* than chicks fed a control diet or a lower concentration of T-2 toxin. These results indicate that diets can affect VFA concentrations, and in some cases, the susceptibility to *Salmonella* colonization. Further research in this area is critical for the successful implementation of an integrated multi-faceted salmonellae prevention and control program in poultry.

IMPACT/TECHNOLOGY TRANSFER F: This research has been presented formally to fellow scientists, poultry industry representatives, and allied industry representatives.

PUBLICATIONS:

Barnhart, E.T., D.J. Caldwell, M.C. Crouch, J.A. Byrd, D.E. Corrier and B.M. Hargis. 1998. Effect of lactose administration in drinking water prior to and during feed withdrawal on *Salmonella* recovery from broiler crops and ceca. Poultry Sci. (in press)

Barnhart, E.T., D.J. Caldwell, M.C. Crouch, J.A. Byrd, D.E. Corrier and B.M. Hargis. 1998. Evaluation of potential disinfectants for pre-slaughter broiler crop decontamination. Poultry Sci. (in press)

Byrd, J.A., D.E. Corrier, J.R. DeLoach, D.J. Nisbet and L.H. Stanker. 1998 Horizontal transmission of *Salmonella typhimurium* in broiler chicks. J. Appl. Poultry Res. 7:75-80.

Byrd, J.A., D.E. Corrier, M.E. Hume, R.H. Bailey, B.M. Hargis and L.H. Stanker. 1998. Incidence of *Campylobacter* in crops of pre-harvest market-age broiler chickens. Poultry Sci. 77:1303-1305.

Byrd, J.A., D.E. Corrier, M.E. Hume, R.H. Bailey, L.H. Stanker and B.M. Hargis. 1998. Effect of feed withdrawal on *Campylobacter* in the crops of market-age broiler chickens. Avian Dis. (in press)

Caldwell, D.J., B.M. Hargis, D.E. Corrier and J.R. DeLoach. 1998. Frequency of isolation of *Salmonella* from protective foot covers worn in broiler houses as compared to drag-swab sampling. Avian Dis. 42:381-384.

Corrier, D.E., J.A. Byrd, B.M. Hargis, M.E. Hume, R.H. Bailey and L.H. Stanker. 1998. Presence of *Salmonella* in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. Poultry Sci. (in press)

Corrier, D.E., J.A. Byrd, M.E. Hume, D.J. Nisbet and L.H. Stanker. 1998. Effect of simultaneous or delayed competitive exclusion treatment on the spread of *Salmonella* in chicks. J. Appl. Poultry Res. 7:132-137.

Corrier, D.E., D.J. Nisbet, J.A. Byrd, B.M. Hargis, N.K. Keith, M. Peterson and J.R. DeLoach. 1998. Dosage titration of a characterized competitive exclusion culture to inhibit *Salmonella* colonization in broiler chickens during grow-out. J. Food Protect. 61:796-801.

Hume, M.E., D.E. Corrier, D.J. Nisbet and J.R. DeLoach. 1998. Early *Salmonella* challenge time and reduction in chick ceca colonization following treatment with a characterized competitive exclusion culture. J. Food Protect. 61:673-676.

Nisbet, D.J., G.I. Tellez, M.H. Kogut, D.E. Corrier, V.K. Lowry, M.L. Hernandez, J.R. DeLoach and L.H. Stanker. 1998. Effect of a commercial competitive exclusion culture PREEMPT™ on mortality and horizontal transmission of *Salmonella gallinarum* in broiler chickens. Avian Dis. (in press)

Sarlin, L.L., E.T. Barhart, D.J. Caldwell, R.W. Moore, J.A. Byrd, D.Y. Caldwell, D.E. Corrier, J.R. DeLoach and B.M. Hargis. 1998. Evaluation of alternative sampling methods for *Salmonella* critical control point determination at broiler processing. Poultry Sci. 77:1253-1257.

ABSTRACTS:

Byrd, J.A., D.E. Corrier, R.H. Bailey and L.H. Stanker. 1998. Effect of defined competitive exclusion culture (PREEMPT™) on cecal colonization and organ invasion by *Salmonella typhimurium* DT104 in broiler chickens. Poultry Sci. 77(Suppl. 1): 95.

Byrd, J.A., D.J. Nisbet, D.E. Corrier and L.H. Stanker. 1997. Use of continuous-flow culture system to study the interation between *Clostridium perfringens* and a mixed microbial competitive exclusion culture CF3™ Biosci. Microflora 16(Suppl.):15.

Hume, M.E., J.A. Byrd, L.H. Stanker and R.L. Ziprin. 1998. Reduction of cecal *Listeria monocytogenes* in Leghorn chicks following treatment with a competitive exclusion culture. Poultry Sci. 77(Suppl. 1):120.

Kubena, L.F., R.H. Bailey, J.A. Byrd, C.R. Young and D.E. Corrier. 1998. Effects of aflatoxins on cecal volatile fatty acids and susceptibility to *Salmonella typhimurium* colonization in broiler chicks. Poultry Sci. 77(Suppl. 1):115.

Kubena, L.F., R.H. Bailey, J.A. Byrd, C.R. Young, D.E. Corrier and L.H. Stanker. 1998. Influence of T-2 toxin and tannic acid on cecal volatile fatty acids and susceptibility to *Salmonella typhimurium* colonization in broiler chicks. Poultry Sci. 77(Suppl. 1):95.

CONTROL OF *SALMONELLA* AND *ESCHERICHIA COLI* O157-H7 IN LIVESTOCK DURING THE PREHARVEST PERIOD

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OBJECTIVE A: To develop rapid diagnostic tests for food-borne pathogens in livestock.

PROGRESS A: Optimized bovine fecal culture methods for enterohemorrhagic *Escherichia coli* (EHEC) and for *Salmonella* were developed. For EHEC O157 detection, conventional bacterial culture, immunomagnetic separation, and colony immunoblot methods were combined with isolate confirmation by ELISA using a panel of anti-O157, anti-EHEC, and anti-H7 monoclonal antibodies (MAbs). An anti-O157 MAb was also used in a blocking ELISA format to accurately detect serum antibodies to *E. coli* O157 in cattle and other livestock. Anti-*E. coli* O26 and anti-*E. coli* O111 MAbs were also developed to permit identification of these important non-O157EHEC serotypes in livestock samples. For *Salmonella* detection in bovine feces, a conventional selection and enrichment culture method was combined with isolate confirmation and sero-grouping/typing by ELISA using a panel of anti-*Salmonella* MAbs.

IMPACT/TECH TRANSFER A: Tech transfer of two anti-EHEC O157 MAbs has been completed via the ImmunoCard Stat! *E. coli* O157:H7 rapid test by Meridian Diagnostics, Inc. Tech transfer of the anti-*Salmonella* MAbs has been initiated.

OBJECTIVE B: To characterize the pre-harvest epidemiology of food-borne pathogens in livestock.

PROGRESS B: Using our optimized EHEC O157 fecal culture method and the blocking ELISA for anti-O157 antibody detection, we identified high fecal EHEC O157 and anti-O157 serum antibody prevalence in Midwest range beef calves at weaning prior to feedlot entry. Out of more than 900 calves sampled from 15 beef herds in five Midwestern states, about 20% of calves shed EHEC O157 in their feces at fall weaning, while about 80% had serum antibodies against *E. coli* O157. Sequential sampling of calves from birth through weaning demonstrated rapid acquisition

of anti-O157 serum antibodies after birth. We also identified four outbreaks of *Salmonella typhimurium* DT104 in beef herds in three states and showed that DT104 appears and disappears rapidly from affected herds.

IMPACT/TECH TRANSFER B: Our studies indicate high prevalence of EHEC O157 in preweaned beef calves, a finding that may have important implications for pre-harvest control of this pathogen.

PUBLICATIONS:

Heaton, M.P., W.W. Laegreid, C.W. Beattie, T.P.L. Smith and S.M. Kappes. 1998. Identification and genetic mapping of bovine chemokine genes expressed in epithelial cells. Mamm. Genome (submitted)

Laegreid, W., M. Hoffman, J. Keen, R. Elder and J. Kwang. 1998. Development of a blocking enzyme-linked immunosorbent assay for detection of serum antibodies to O157 antigen of *Escherichia coli*. Clin. Diagn. Lab. Immunol. 5(2):242-246.

Siragusa, G.R., W.J. Dorsa, C. N. Cutter, G.L. Bennett, J.E. Keen and M. Koohmaraie. 1998. The incidence of *Escherichia coli* on beef carcasses and its association with aerobic mesophilic plate count categories during the slaughter process. J. Food Protect. (in press)

ABSTRACTS:

Elder, R.O., W.W. Laegreid, R.B. Westerman, J.E. Keen, J. Kwang and R. Wilson. 1997. A common surface epitope shared by enterohemorrhagic *Escherichia coli*. 78th Conf. Res. Anim. Dis., Chicago, IL, 29.

Grosse, W.M., M.P. Heaton, S.M. Kappes, J.W. Keele and W.W. Laegreid. 1998. Development of single nucleotide polymorphism (SNP) markers for bovine C-X-C chemokine genes. 79th Conf. Res. Anim. Dis., Chicago, IL. (submitted)

Heaton, M.P., C.W. Beattie and W.W. Laegreid. 1997. Gene expression in bovine epithelial cells: identification of chemokine genes induced by *Escherichia coli* O157:H7 lipopolysaccharide. 78th Conf. Res. Anim. Dis., Chicago, IL, 18.

Keen, J.E., R. Snell, Y. He and W.W. Laegreid. 1997. Salmonellosis outbreak and subsequent subclinical infection in a Nebraska beef herd attributable to clonal *Salmonella* isolates of identical PFGE genotype but variable serologic phenotype. 78th Conf. Res. Anim. Dis., Chicago, IL, 85.

Keen, J.E. and W.W. Laegreid. 1997. Transmission of *Salmonella typhimurium* ssp. *copenhagen* from a chronic fecal shedding beef cow to naive herdmates. 1997. 78th Conf. Res. Anim. Dis., Chicago, IL, 86.

Keen, J.E., T. He, E.K. Daniels and N.E. Woollen. 1998. Optimized bovine fecal culture for Salmonellae with isolate confirmation and serogrouping/typing by an anti-*Salmonella* monoclonal antibody panel. 79th Conf. Res. Anim. Dis., Chicago, IL. (submitted)

Laegreid, W.W., J. Keen and J. Kwang. 1997. Development of a blocking enzyme-linked immunosorbent assay for detection of serum antibodies to O157 antigen of *Escherichia coli*. 78th Conf. Res. Anim. Dis., Chicago, IL, 89.

Laegreid, W.W., R.O. Elder and J.E. Keen. 1998. Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. 79th Conf. Res. Anim. Dis., Chicago, IL. (submitted)

Love, B.C., C.L. Taylor, M. Michalak, J.E. Keen, T.E. Wittum and B.A. Byrum. 1998. *Salmonella typhimurium* DT104 in a swine finishing operation in Ohio. 79th Conf. Res. Anim. Dis., Chicago, IL. (submitted)

Rivera, M. and J.E. Keen. 1998 Monoclonal antibodies specific for *Escherichia coli* serotypes O26 and O111. 79th Conf. Res. Anim. Dis., Chicago, IL. (submitted)

PREVENTION OF LOSSES FROM COLIBACILLOSIS AND *ESCHERICHIA COLI* O157:H7 IN CATTLE AND SWINE

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OBJECTIVE A: Identify site and mechanism of O157:H7 colonization in cattle and identify other virulence attributes of O157:H7 and Shiga toxin-producing *Escherichia coli.*

PROGRESS A: Cattle are a source of *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC) which causes foodborne diseases in humans including bloody diarrhea, severe kidney disease, and sometimes death. Identifying the site and mechanism of STEC colonization is the first step in reducing the amount of STEC in the intestinal tract and in feces. This should decrease the incidence of human disease caused by consumption of contaminated beef.

We have shown that *E. coli* O157:H7 cause severe, sometimes fatal, disease in newborn calves (< 12 h old). Newborn calves develop diarrhea, have intestinal damage, and shed high levels of O157:H7 as early as 18 h after experimental infection with *E. coli* O157:H7. *E. coli* O157:H7 do not cause disease in weaned calves (3-4 months old). Weaned calves that were fasted 48 h prior to inoculation with *E. coli* O157:H7 had intestinal damage and shed high levels of O157:H7 (relative to control *E. coli*) at 4 d postinoculation (see Objective B).

We have developed and are using experimental infection models in neonatal calves, weaned calves, and neonatal pigs to characterize the role of specific bacterial genes in colonization and pathogenesis of O157:H7 and other Shiga toxin-producing *E. coli* in bovines. Two *E. coli* O157:H7 genes have been identified which are believed to contribute to colonization and pathogenesis. We found that intimin, but not Stx, was required for *E. coli* O157:H7 pathogenesis (symptomatic infection) in neonatal calves. Both intimin and Stx promoted intestinal colonization in weaned calves. We have also shown that a human pathogenic STEC isolate, which does not express intimin and is not O157:H7, colonized and caused brain lesions similar to those caused by O157:H7 in neonatal pigs. This suggests that intimin and Stx may not be the only STEC genes that are important for colonization and pathogenicity.

IMPACT/TECH TRANSFER A: These results show that Stx and intimin are potential targets for intervention strategies to reduce *E. coli* O157:H7 infections and shedding in cattle. Reducing the levels of *E. coli* O157:H7 in cattle should reduce the risk of *E. coli* O157:H7 infections in humans. These results have led to collaborative studies with Dr. A. D. O'Brien (Uniformed Services University of Health Sciences, Bethesda, MD) to determine if anti-intimin antibodies will reduce O157:H7 shedding (see Objective B). The results of experimental infection with the non-O157:H7 STEC strain that does not express intimin suggest that there are other colonization mechanisms in STEC and that these may also be targets for preventing colonization and fecal shedding in cattle. This work led to an invited presentation (by E. A. Nystrom) at the Rowett Research Institute, Aberdeen, Scotland, in a workshop entitled "Farm animals as reservoirs for *Escherichia coli* O157:H7."

OBJECTIVE B: Identify methods to reduce shedding of O157:H7 in cattle.

PROGRESS B: The results of Objective A demonstrate that intimin is required for O157:H7 colonization and pathogenicity. Preliminary experiments are being conducted to determine if anti-intimin antibody will passively prevent or reduce colonization and disease in neonatal pigs inoculated with O157:H7 *E. coli*.

A second approach to developing methods to reduce shedding of *E. coli* O157:H7 in cattle is to examine the gastrointestinal ecology of *E. coli* and identify specific conditions which cause increased fecal shedding of O157:H7 and other coliforms in cattle. Previous work has shown that *E. coli* is inhibited by the low pH and high volatile fatty acid concentration in the rumen of well fed cattle. We have shown that fasting decreases the pH and volatile fatty acid concentration in the rumen and this can allow the growth of coliforms and *E. coli* O157:H7. We tested the hypothesis that fasting stress would cause increased fecal shedding of O157:H7 *E. coli* in calves. We found that fasted calves were more susceptible to infection and shed higher numbers of O157:H7 *E. coli* than normally fed calves (see Objective A). However, fasting did not increase O157:H7 shedding in previously infected calves.

In addition to fasting, other types of stress may cause increased fecal shedding of *E. coli* in cattle. We collected fecal samples from pregnant dairy cows to determine if the stress of calving would cause increased coliform shedding. We found that there was a sharp increase in coliform shedding within 12 days of calving. In contrast, earlier samples from prepartum cows had very low or undetectable numbers of fecal coliforms.

IMPACT/TECH TRANSFER B: The results demonstrate that fasting increases the susceptibility of calves to *E. coli* O157:H7 infection and shedding and suggest that maintaining a regular feeding schedule may decrease susceptibility and reduce fecal shedding. Other stresses, such as calving, may also increase fecal shedding of *E. coli*. Other intervention strategies, including passive protection using anti-intimin antibody to prevent or reduce O157:H7 colonization and shedding, are in progress.

OBJECTIVE C: Develop rapid methods to identify and quantify *E. coli* O157:H7 and other STEC pathogenic for humans in tissues and fluids from cattle, and develop a rapid method to detect fecal contamination on meat samples.

PROGRESS C: A multiplex PCR method which simultaneously detects enterotoxigenic, attaching and effacing, and Shiga toxin-producing (both Stx1 and Stx2) *E. coli* strains from calves was developed in collaboration with Dr. H. Moon and S. Franck at Iowa State University (ISU).

Although specific methods for identifying foodborne pathogens are important, additional technology is needed to detect fecal contamination to support the FSIS zero-tolerance rule. In collaboration with Dr. Jacob Petrich at ISU, we have developed a method and device which detects fecal contamination on carcasses in near real-time. We have built a working hand-held prototype which is sensitive and specific for feces. This prototype successfully detected fecal contamination on meat samples collected at a packing plant. We are building additional prototypes which are designed to be portable or which will image whole carcasses for fecal contamination.

IMPACT/TECH TRANSFER C: Method 1 - The multiplex PCR method is being used in the Iowa State University diagnostic laboratory to identify, differentiate, and characterize pathogenic *E. coli* isolates from calves. The results will be useful to producers and veterinarians for the preliminary diagnosis of diseases caused by *E. coli* in calves.

Method 2 - A U.S. patent was filed (March 1998) on the fecal detection device that we have invented. We expect that ARS and ISU will soon decide if foreign filing will also be initiated. This device will be useful in the meat packing industry to detect and prevent fecal contamination and human foodborne pathogens from entering the food supply. There have been over 50 contacts with interested organizations including meat packers, instrument manufacturers, and the media. The technology was disclosed under confidentiality agreement with approximately 30 different firms and detailed presentations to approximately 20 of these. The purpose of these disclosures is to identify corporate partners/sponsors that are capable of producing and marketing a commercial device that is useful to industry. We expect to have a CRADA with a corporate partner within three months and a prototype to test in packing plants within the next 6-12 months.

PUBLICATIONS:

Cray Jr., W.C., T.A. Casey, B.T Bosworth and M.A. Rasmussen. 1998. Effect of dietary stress on fecal shedding of *Escherichia coli* O157:H7 in calves. Appl. Environ. Microbiol. 64:1975-1979.

Dean-Nystrom, E.A., B.T. Bosworth, H.W. Moon and A.D. O'Brien. 1998. Bovine infection with STEC, p. 261-267. *In:* A. D. O'Brien and J. B. Kaper (eds.), *E. coli* O157:H7 and other shiga toxin-producing *E. coli* strains. ASM Press, Washington, D.C.

Dean-Nystrom, E.A., B.T. Bosworth, H.W. Moon and A.D. O'Brien. 1998. *Escherichia coli* O157:H7 requires intimin for enteropathogenicity in calves. Infect. Immun. 66:4560-4563.

Franck, S.M., B.T. Bosworth and H.W. Moon. 1998. Multiplex PCR for enterotoxigenic, attaching and effacing, and shiga toxin-producing *Escherichia coli* strains from calves. J. Clin. Microbiol. 36:1795-1797.

Pelan-Mattocks, L.S., M.E. Kehrli Jr., T.A. Casey and J.P. Goff. 1998. Variable shedding of fecal coliform bacteria from periparturient dairy cows. Am. J. Vet. Res. (in press)

ABSTRACTS:

Ackerman, M., E. Dean-Nystrom, S. Jones, A. Florance and H. Moon. 1997. Semi-quantitation of neutrophil infiltration into intestinal mucosa in a neonatal calf model of *E. coli* O157:H7 enteritis. Am. Coll. Vet. Pract.

Cornick, N.A., T.A. Casey and H.W. Moon. 1997. Competitive colonization of sheep by *E. coli* O157:H7. Conf. Rumen Function, Chicago, IL, 36.

Dean-Nystrom, E.A., B.T. Bosworth and H.W. Moon. 1998. Pathogenesis of *Escherichia coli* O157:H7 in weaned calves. 2nd Int. Rushmore Conf. Mechan. Pathog. Enteric Dis., Rapid City, SD.

Dean-Nystrom, E.A., B.T. Bosworth, A.D. O'Brien and H.W. Moon. 1998. Bovine infection with *Escherichia coli* O157:H7. Int. Workshop Farm Anim. Reserv. *Escherichia coli* O157, Aberdeen, Scotland, 8.

O'Brien, A.D., L.J. Gansheroff, M.R. Wachte, H.W. Moon, B.T. Bosworth, C.N. Stewart and E. A. Dean-Nystrom. 1998. Intimin: Candidate vaccine to prevent colonization of cattle by *Escherichia coli* O157:H7. Annu. Can. Soc. Microbiol. Meet. 49.

Rasmussen, M.A., T.L. Wickman, W.C. Cray Jr., and T. A. Casey. 1998. *Escherichia coli* O157:H7 and the rumen environment. Int. Workshop Farm Anim. Reserv. *Escherichia coli* O157, Aberdeen, Scotland, 6-7.

CONTROL OF *CAMPYLOBACTER JEJUNI* IN POULTRY

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OBJECTIVE A: Develop immunity-based intervention strategies against *Campylobacter* colonization of chickens.

PROGRESS A: *Campylobacter* naturally resides in chickens without causing an immune response that clears the organism. We have discovered that *Campylobacter* produces a substance that induces cell death of chicken lymphocytes. The substance appears to be active against all lymphocytes, but regulatory T-cells are particularly susceptible. We have begun the process of purifying and identifying the active component of *Campylobacter* responsible for the activity. It appears to be a protein that segregates with the outer membrane and is not flagellin. Studies are underway to determine if the substance is active *in vivo*.

IMPACT/TECH TRANSFER A: It appears that *Campylobacter* has an active mechanism of immune-avoidance. To succeed with a vaccine against *Campylobacter* will probably require countering that mechanism. This study is the beginning of the process to understand the mechanisms used by *Campylobacter* so that new vaccines can be designed that have a chance of success.

OBJECTIVE B: Evaluation of the diversity of *Campylobacter jejuni*.

PROGRESS B: Members of the *C. jejuni* species are extremely variable. Some of the variability may reflect immune-avoidance, but some may also reflect host preferences by strains. Conclusions about the cause of the variability of *Campylobacter* and the relationships of strains require the combined evaluation of several parameters. Each individual parameter can stand alone as a typing method. We have extended the DNA sequence based typing system to cover more strains. A project is underway to compare several typing systems. Data from a multi-locus enzyme

electrophoresis study of a large population of *Campylobacter* has been analyzed and it was found that portions of the chromosome of the organism do reflect the environment from which the strain was obtained. Conclusions on what drives the diversity still can not be made. We have compared the diversity of two related genes that are found closely spaced on the chromosome.

IMPACT/TECH TRANSFER B: These studies will help improve methods for epidemiological study of *Campylobacter*. The Enterics Laboratory of the Centers for Disease Control, National Center for Infectious Disease, has decided to investigate the DNA sequence-based typing system developed in our laboratory for use as a standard method.

OBJECTIVE C: Develop methods for detection, enumeration and recovery of *Campylobacter*, as well as evaluation of current cultural methods to detect *Campylobacter* from dry samples commonly examined in poultry production.

PROGRESS C: Experiments were designed and conducted to determine optimum methods for enumeration of *Campylobacter* in poultry carcasses. The 8 experiments conducted involved over 3400 *Campylobacter* analyses. The results of these trials and the 6 experiments conducted during FY97 will be used to establish a new procedure for *Campylobacter* enumeration to be published in the FSIS Microbiology Laboratory Guidebook. A study was completed whereby known numbers of *C. jejuni* were inoculated onto chick pads, unused pine shavings (litter), and eggshells. The ability to recover cells from these samples eroded dramatically over a 30 to 60 min period.

IMPACT/TECH TRANSFER C: Our studies provide an efficient and valuable method for the detection and enumeration of *Campylobacter* from freshly processed poultry carcasses, thus allowing for characterization of the public exposure to this pathogen. Further, our results show that if dry samples are not examined immediately following contamination, the results may not be accurate.

OBJECTIVE D: Use applied interventions in the spread of *Campylobacter* within the poultry production environment.

PROGRESS D: Research was begun in a new area involving the application of poultry litter amendments to reduce *Salmonella* and *Campylobacter* populations in broilers. These acidifying litter treatments are intended for ammonia reduction but have proven efficacious in reducing pathogen colonization of poultry in our floor pens. In preliminary floor pen trials involving more than 960 broilers both *Salmonella* and *Campylobacter* populations were reduced. Confirmatory pen trials are in progress. In a separate field trial conducted with a major poultry integrator (utilizing one litter treatment) we analyzed over 2200 samples for *Campylobacter* during a 3 month period. We found no effect from the application of poultry litter in the field study investigated.

IMPACT/TECH TRANSFER D: None

OBJECTIVE E: Determine the extent of *Campylobacter* contamination of turkeys in commercial production.

PROGRESS E: Four commercial turkey flocks were examined for presence of *Campylobacter* by examination of droppings at ages of 3, 6, 9 and 12 weeks. *Campylobacter* contamination was noted in all flocks at levels ranging from 63 to 87% throughout production.

IMPACT/TECH TRANSFER E: The onset of *Campylobacter* contamination in turkeys occurred at a different time and different levels than that noted in broilers indicating intervention strategies may need to be developed specifically for turkeys.

PUBLICATIONS:

Line, J.E., J.S. Bailey, N.J. Stern, and T.A. Tompkins. 1998. Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. Poultry Sci. 77(suppl. 1):405-410.

ABSTRACTS:

Berrang, M.E., N.A. Cox, S.E. Craven and N.J. Stern. 1998. Effect of sub-therapeutic levels of antibiotics in feed on the intestinal carriage of *Campylobacter* and *Salmonella* in turkeys. Poultry Sci. 77 (suppl. 1):96.

Berrang, M.E., N.A. Cox, M.T. Musgrove and N.J. Stern. 1998. Recovery of *Campylobacter* from dry poultry associated samples. Poultry Sci. 77 (suppl. 1):96.

Chen, H.C. and N. J. Stern. 1998. Competitive exclusion of heterologous *Campylobacter* spp. in chicks. Annu. Amer. Soc. Microbiol. P-88.

Chen, H.C. and N.J. Stern. 1998. Comparison of two molecular techniques for epidemiological tracing of *Campylobacter jejuni*. Annu. IAMFES Meet. 94.

Cox, N.A., N.J. Stern, M.T. Musgrove and M.E. Berrang. 1998. Prevalence of *Campylobacter* in the cecal droppings of turkeys during production. Poultry Sci. 77 (suppl. 1):113.

Hiett, K.L. and R.J. Meinersmann (1998) Phylogenetic analysis of two physically linked genes in *Campylobacter*. Annu. Amer. Soc. Microbiol. 486.

Line, J.E. 1998. Alum treatment of poultry litter to control *Salmonella* and *Campylobacter*. Annu. Meet Center Food Saf. Qual. Enhan.

Line, J.E., N.J. Stern, C.P. Lattuada and S.T. Benson. 1998. *Campylobacter* recovery and enumeration from broiler carcasses. Annu. IAMFES Meet.

Line, J.E. 1998. The Federal Research Agenda for fresh fruit and vegetable safety. Symp. Fresh Fruits and Veg. Food Saf. Chall., NCFST/IFT.

Line, J.E. 1998. Aluminum sulfate treatment of poultry litter to reduce *Salmonella* and *Campylobacter* populations. Poultry Sci. 77 (Suppl. 1): 96.

Line, J.E. 1998. Food safety issues concerning animal manure, waste utilization and management. ARS Nat. Prog. Staff Workshop Anim. Manure Waste Utiliz. Kansas City, MO.

Musgrove, M.T., N.J. Stern, N.A. Cox and M.E. Berrang. 1998. Discrimination between *Arcobacter* spp. And *Campylobacter* spp. Isolated from enriched broiler carcass rinses. Poultry Sci. 77 (suppl. 1):113.

Patton, C.M. , G.M. Evins, B.D. Plikaytis, I.K. Wachsmuth, P.I. Fields and R.J. Meinersmann. (1998) Genetic diversity and relationships among *Campylobacter* species and subspecies. Annu. Amer. Soc. Microbiol. 111.

Stern, N.J., N.A. Cox, J.S. Bailey, S.E. Craven, J.E. Line and R.J. Meinersmann. 1997. *Salmonella* and *Campylobacter* epidemiology and control during broiler production. Proc. World Cong. Food Hyg., Hague, Netherlands.

Thompson, S.A., R.J. Meinersmann, E. Pigman, Y. Li, K.C. Ray and M.J. Blaser. 1998. Genetic diversity of *Helicobacter pylori* isolated from geographically separate locations. Annu. Amer. Soc. Microbiol. 112.

Zhu, J., R. Meinersmann, K. Hiett and D. Evans. (1998) Apoptotic effect of outer membrane proteins from *Campylobacter jejuni* on *in vitro* cultured chicken lymphocytes. 19th Annu. SSPS Meet.

Zhu, J., R. Meinersmann, K. Hiett and D. Evans. (1998) Response of chicken lymphocytes to outer membrane proteins of *Campylobacter* jejuni. Poultry Sci. 77(suppl. 1):40

PREVENTION IN LIVESTOCK OF POTENTIAL HUMAN FOODBORNE PATHOGENS

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OBJECTIVE A: To develop rapid and sensitive DNA-based techniques to detect and to differentiate *Campylobacter*, and *Campylobacter*-like organisms in food and livestock.

PROGRESS A: Members of the genus *Campylobacter* are major causes of human enteritis following consumption of contaminated food, water, or milk. Since there are few laboratory tests to differentiate these microbes, we have developed rapid assays based on the polymerase chain reaction (PCR) to aid in their identification. We have previously described a multiplex PCR to differentiate the thermotolerant *C. jejuni* and *C. coli*. Enrichment of *Campylobacter* typically requires blood-based media, incubation in a low oxygen environment, and multiple incubation temperatures to maximize recovery of injured cells. We have evaluated two blood-free media, Rosef's and Tran's, for the recovery of *Campylobacter* from poultry cloacal and hog fecal samples. Based on ease of preparation and recovery of *Campylobacter*, Tran's formulation, developed by FDA, was found to be the most efficient. It is a blood-free medium and does not require any special gas atmosphere for recovering *Campylobacter*. Enrichment in Tran's combined with the multiplex PCR assay, which differentiates *C. jejuni* from other thermotolerant *Campylobacter* species, was used to detect *Campylobacter* in hog fecal samples. Pigs from four farms each in Iowa (n = 2 40) and North Carolina (n = 240) were sampled at the nursery stage through to slaughter. All-in and all-out as well as continuous flow production farms were analyzed. At slaughter, carcass swabs and ileocecal lymph nodes were cultured. *Campylobacter* was readily detected in > 90% of fecal swabs obtained at the nursery, grower and finisher stages, and the day prior to slaughter. After slaughter, *Campylobacter* was detected in 9% of carcass swabs and from 83% of the ileocecal lymph nodes of Iowa hogs.

Arcobacter spp. are aerotolerant *Campylobacter*-like organisms which have been recovered from livestock and meats, and have been associated with human enteritis. Of the four species of *Arcobacter, A. butzleri* is regarded as the human pathogen. *Arcobacter*, like *Campylobacter*, has been reported more frequently from poultry than from red meats, with recoveries ranging from 24 to 81% of poultry carcasses. In this study we sampled turkey products obtained from three packing

plants. *Arcobacter* was detected in 77% of mechanically separated turkey samples. Overall, *A. butzleri* was cultured from 56% of the samples examined. All isolates were confirmed by DNA probes. Differences in recovery rates were seen from samples obtained from plants A (77%), B (46%), and C (26%). This is analogous to the previously reported plant-to-plant variation which we observed in the recovery of *Arcobacter* spp. from ground pork. Genetic analysis of 121 isolates yielded 86 patterns, indicating multiple sources of contamination.

A. butzleri is frequently recovered from retail purchased poultry. To determine the incidence of natural infection, we screened nearly 400 cloacal swabs by multiplex PCR. *Arcobacter* spp. and *A. butzleri* were detected in 15% and 1% of birds, respectively. We have been unable to experimentally infect conventional three-day-old chicks (n = 41) or five-day-old turkey poults (n = 67) with *A. butzleri*. In contrast, 65% of the highly inbred three-day-old Beltsville White turkeys (n = 106) could be colonized. This indicates that multiple factors, including genetic susceptibility, may be needed to establish natural infections of *Arcobacter* in birds.

IMPACT /TECH TRANSFER A: These assays will contribute to the rapid identification of these fastidious pathogens and thus provide a better estimate of their role in causing human foodborne illness. These assays are simple to perform and thus are well suited for large scale field studies.

OBJECTIVE B: To adapt rapid methods to detect *Listeria* in food and livestock.

PROGRESS B: Human outbreaks of *Listeria monocytogenes* have been associated with consumption of contaminated poultry products, including turkey frankfurters. In a continuation of a field study reported last year, we adapted a multiplex PCR assay to detect *Listeria* and *L. monocytogenes* in mechanically separated turkey meat samples (n = 150). Overall, *Listeria*, including *L. monocytogenes*, was isolated from 84% of the meat samples (126/150). *L. monocytogenes* from 49% of the samples (73/150). Isolates were identified by serotyping as type 1 (71% %), type 4 (27%), and nontypable (2%).

The multiplex PCR assay has also been used to confirm *L. monocytogenes* in a case of fatal listeriosis in sheep. *L. monocytogenes* was recovered from soil, water, and compost piles sampled and confirmed by the multiplex PCR. The clinical and environmental isolates were identified as serotype 1. Analysis indicated that the environmental and clinical sheep isolates were distinctly different. This study indicates the ease of coupling PCR-based assays for the identification and molecular fingerprinting of *L. monocytogenes*.

IMPACT/TECH TRANSFER B: These results indicate the suitability of PCR-based methods to identify potential human foodborne pathogens in livestock and in foods. The assays are highly reproducible and provide definitive identification in a shorter time than conventional biochemical testing.

OBJECTIVE C: To adapt PCR-based methods to the detection of *Yersinia* in livestock.

PROGRESS C: Swine have been implicated as the principal reservoir of human pathogenic *Yersinia enterocolitica*, with the prevalence in pork tissues reported to be as high as 96.5%. We utilized a PCR assay to estimate the prevalence of *Y. enterocolitica* in pigs obtained from eight different sites in Iowa and North Carolina. For each state, half of the farms used the all-in all-out production system and half used continuous flow production units. Fecal samples and tonsils were placed in Irgasin-ticarcillin-potassium chlorate (ITC) broth and DNA extracted for PCR detection. The assay targeted the *ail* gene, which is a chromosomally-encoded virulence factor unique to *Y. enterocolitica*. None of the tonsil or fecal samples from Iowa pigs (n = 240) and only 2% of tonsillar swabs from North Carolina pigs (n = 240) were positive. In contrast, 42% of tonsils obtained from Pennsylvania (n = 60) yielded *Yersinia*. This may indicate that there is a decided regional difference in the distribution for *Y. enterocolitica*.

IMPACT/TECHNOLOGY TRANSFER C: Development of a rapid PCR-based assay will be helpful in screening field samples. Results from this study will be useful in correlating the distribution of foodborne pathogens with on-farm management practices.

PUBLICATIONS:

Manke, T.R., I.V. Wesley, J.S. Dickson and K.M. Harmon. 1998. Prevalence and genetic variability of *Arcobacter* and *Arcobacter butzleri* in mechanically separated turkey. J. Food Protect. (in press).

Oliveira, S. J. de, A.L. Baetz, I.V. Wesley and K.M. Harmon. 1997. Classification of *Arcobacter* species isolated from aborted pig fetuses and sows with reproductive problems in Brazil. Vet. Microbiol. 57:347-354.

Oliveira, S.J. de, I.V. Wesley, A.L. Baetz, K.M. Harmon, I.T. Ivonyr and M. de Uzeda. 1998. *Arcobacter cryaerophilus* and *Arcobacter butzleri* isolated from preputial fluid of boars and fattening pigs in Brazil. J. Vet. Diagn. Invest. (submitted)

Oyarzabal, O.A., I.V. Wesley, J.M. Barbaree, L.H. Lauerman and D.E. Conner. 1998. Identification and characterization of *Arcobacter* and *Campylobacter* from retail poultry by physiological testing and the polymerase chain reaction technique. Poultry Sci. (submitted)

Oyarzabal, O.A., I.V. Wesley, K.M. Harmon, L. Schroeder-Tucker, J.M. Barbaree, L.H. Lauerman, S. Backert and D.E. Conner. 1997. Specific identification of *Campylobacter fetus* by PCR targeting variable regions of the 16S rDNA. Vet. Microbiol. 58:61-72.

Rice, W.E., M.R. Rodgers, I.V. Wesley, C,H, Johnson and A.S. Tanner. 1998. Isolation of *Arcobacter* spp. from ground water. J. Appl. Microbiol. (submitted)

Suarez, D.L., I.V. Wesley and D. Larson. 1997. Detection of *Arcobacter* species in gastric samples from swine. Vet. Microbiol. 57:325-336.

Wesley, I.V. 1997. *Helicobacter* and *Arcobacter* - Potential human foodborne pathogens? Trends Food Sci. Technol. 8:293-299.

Wesley, I.V. 1998. Listeriosis infection in animals, pp. 39-74. *In:* E. Marth and E. Ryser (eds.), *Listeria*, Listeriosis and Food Safety. Marcel Dekker, NY.

Wesley, I.V. 1998. *Arcobacter. In:* Encyclopedia Food Microbiology, Academic Press. (in press)

Wesley, I.V. 1998. *Helicobacter. In:* Encyclopedia Food Microbiology, Academic Press. (in press)

Wesley, I.V., and A.L. Baetz. 1998. Natural and experimental infection of birds with *Arcobacter butzleri.* Poultry Sci. (submitted)

Wesley, I.V., and S.C. Johnson. 1997. Detection of *Yersinia enterocolitica* in pigs and pork products. Swine Res. Rep., Iowa State Univ. Exten. 106-113.

Wesley, I.V., T.P. Sanderson, D.J. Larson, K.M. Harmon, J.J. Andrews, D.W. Miskimins, D.H. Zeman. 1997. Application of multiplex PCR for rapid identification of *Campylobacter jejuni* and *C. coli* associated with reproductive failure. Amer. J. Vet. Res. 58:1070-1075.

ABSTRACTS:

Ramos, A.M., I.V. Wesley, J.M. Dickson and K.M. Harmon. 1997. Rapid detection of *Listeria monocytogenes* in mechanically separated turkey meat. Proc. Annu. Meet. Food Safety Consortium., 220-224.

Wesley, I.V. 1997. *Campylobacter* and related microorganisms in cattle. Amer. Meat Sci. Inst. Beef Safety Symp., Chicago, IL, 30-41.

Wesley, I.V. 1998. *Campylobacter*, *Helicobacter* and *Yersinia* - What do we know? Nat. Pork Prod. Summit, Des Moines, IA, 194-204.

Wesley, I.V. 1998. Association and incidence of *Arcobacter*, *Helicobacter*, and *Campylobacter* in pork. Annu. IAMFES Meet., 80.

Wesley, I.V., and A.L. Baetz. 1998. Experimental infection of birds with *Arcobacter butzleri*. Annu. Amer. Soc. Microbiol., 121.

Wesley, I.V., and A.L. Baetz. 1998. Experimental infection of birds with *A. butzleri*. Annu. IAMFES Meet., 64.

Wesley, I.V., and K.M. Harmon. 1997. A multiplex PCR method to detect *Campylobacter jejuni*, *C. coli* and *Arcobacter* in livestock. 9th Int. Workshop *Campylobacter*, *Helicobacter* and related organisms, 57.

DISEASE RELATED PROBLEMS OF POULTRY PRODUCTION AND PROCESSING (OSTEOMYELITIS IN TURKEYS, PROVENTRICULITIS IN BROILERS, AND INTESTINAL STRENGTH)

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OBJECTIVE A: To determine the etiology of turkey green-liver/osteomyelitis complex (TOC), evaluate the involvement of the immune system in TOC, and to develop methods to reduce the impact of TOC on turkey production.

PROGRESS A: USDA-ARS research on TOC is focused on determining why a small percentage of healthy-appearing processed turkeys are infected with a variety of opportunistic bacteria, affecting bone, muscle, and synovial tissue. Previous studies conducted by this group suggest that immunological dysfunction contributes to the onset of TOC. We have developed a model that reproduces all aspects of TOC. This model uses intramuscular injection of dexamethasone and air sac inoculation of *Escherichia coli* at 5 weeks of age, followed by a second dexamethasone injection. Dexamethasone is a synthetic corticosteroid which mimics the stress response and is known to suppress immune function. We hypothesize that differences in the stress response of individual birds and resultant immunodeficiencies may be related to their susceptibility to these opportunistic infections. This is supported by the fact that TOC is only a problem in male turkeys, rarely in females, and the endocrine alterations involved in stress are influenced by gender. Work this year has provided additional support that TOC develops in turkeys that are immuno-compromised and supports the hypothesis that the cause of TOC is either an inherent and/or stress induced immunodysfunction.

IMPACT/TECH TRANSFER A: Our ability to experimentally reproduce the lesions of TOC is important for four reasons. First, field incidence of TOC is only 0.5%, a level too low for statistical evaluation of the effect of remedial measures such as antibiotic treatment or immunomodulation. This model should enable us to evaluate such measures. Second, this model is the first to demonstrate the possibility of a respiratory origin for the bacteria that cause TOC. Third, these data suggest that TOC might result from immune dysfunction and thus might be prevented by immunomodulation, and fourth, since *E. coli* air sacculitis/septicemia is considered by some to be

the most important turkey disease, this model may have wide impact on turkey health. This research could lead to the reduction of TOC in turkeys, which would consequently reduce the need for the FSIS inspection procedures to identify affected carcasses. We are currently investigating methods of reducing stress and enhancing the immune responsiveness on the development of TOC.

OBJECTIVE B: To isolate and characterize the etiological agent of proventriculitis in broilers

PROGRESS B: Proventriculitis is a problem of food safety significance because rupture of the proventriculus during processing causes carcass contamination with intestinal contents. We have established that homogenates of affected proventricular material will cause proventriculitis when fed to day-old broilers. Progress has been made on the isolation and characterization of viruses and bacteria found in affected proventriculi. Work this year has found that when a bacteria isolate and a suspect viral agent are used to reproduce this disease, there is an interaction between these agents that causes severe proventriculitis. These studies suggests that this disease is not caused by a single agent but results from a variety of insults to the proventriculus.

IMPACT/TECH TRANSFER B: We are co-investigators on a patent application for development of a vaccine to prevent proventriculitis using IBDV isolated from proventriculus homogenates. Current work is targeted at further characterizing the agents responsible for proventriculitis.

OBJECTIVE C: To develop methods to increase intestinal strength of poultry.

PROGRESS C: Intestines can become weakened and easy to tear due to the effects of disease, mycotoxins, and diet. Mechanical evisceration can then result in torn intestines which contaminate carcasses and increase the spread of potential pathogens throughout the processing plant. We have developed a sensitive method for accurately measuring intestinal strength not only to document the effects of agents which decrease intestinal strength, but also to evaluate ways to increase intestinal strength. We have evaluated many approaches to increasing intestinal strength such as feeding short chain fatty acids like propionic acid, using the astringents tannic acid and alum, and trying to buffer the intestinal tract with calcium carbonate. To date, these approaches have proven to be ineffective in increasing intestinal strength. Preliminary data collected this year on the use of β-glucan to increase intestinal strength suggests that this is also not effective in increasing intestinal strength.

IMPACT/TECH TRANSFER C: The methodology developed for measuring intestinal strength is a valuable tool for research into intestinal disease. Development of methods to increase intestinal strength would decrease the cost of poultry processing and increase product safety. We are using this technology to continue to search for a practical way to increase intestinal strength in poultry.

PUBLICATIONS:

Huff, G.R., W.E. Huff, J.M. Balog and N.C. Rath, 1998. The effects of dexamethasone immunosuppression on turkey osteomyelitis complex in an experimental *Escherichia coli* respiratory infection. Poultry Sci. 77 (suppl. 1):654-661.

ABSTRACTS:

Huff, G.R., W.E. Huff, J.M. Balog, N.C. Rath and Q. Zheng, 1997. Studies on infectious proventriculitis. Proc. 32nd Nat. Meet. Poultry Health Proc., Ocean City, MD, 39-43.

DEVELOPMENT OF MICROBIAL COMPETITIVE EXCLUSION METHODS TO REDUCE PATHOGENIC BACTERIA IN SWINE

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FSIS CODE: C1AM04
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OBJECTIVE A: Develop a competitive exclusion culture to prevent and/or control *Salmonella* in swine and identify critical control points where competitive exclusion or other appropriate intervention strategies can be incorporated to control enteropathogens during commercial production of swine.

PROGRESS A: Several competitive exclusion cultures have been established in continuous-flow culture and these have been evaluated for efficacy using *in vitro* and *in vivo* protocols developed and validated in our laboratory. Differences in efficacy were observed between cultures derived from different pigs. Two of these cultures have been selected for further development based on their superior ability to enhance colonization resistance to *Salmonella* species of baby pigs. Furthermore, this protection is maintained beyond the weaning period. These results are pertinent to our finding that weaning appears to facilitate the rapid spread of *Salmonella* throughout modern swine production systems.

IMPACT/TECH TRANSFER A: Patent protection for these porcine derived competitive exclusion cultures is pending. A partnership has been formalized via a Cooperative Research and Development Agreement (CRADA) between our laboratory and an industry partner and a joint investigational new animal drug application is being prepared for review by the FDA. A postdoctoral scientist funded through the CRADA has been working in our laboratory on development of practical methods of product administration and has contributed to the development of commercial scale-up protocols which are now being implemented by the CRADA partner. Communication of our findings to the scientific community and to producers remains a high priority. Subsequently, we have presented our findings at national and international meetings and have submitted several reports to reputable scientific journals; some of these have now been published. Moreover, several integrated swine producers have expressed interest in competitive exclusion and we have presented results of our work at their requests. Collaborations have been formalized with some of these producers.

OBJECTIVE B: Characterize the microbial consortiums making up the competitive exclusion cultures and elucidate mechanistic aspects of competitive exclusion technology.

PROGRESS B: Characterization of the microbial composition of one porcine-derived competitive exclusion culture by traditional culture methods is complete; and similar characterization of another is nearly complete, as evidenced by analysis of genetic diversity. A molecular-based protocol is now being used to characterize competitive exclusion cultures that show differing abilities to control *Salmonella in vitro* and *in vivo*. Certain members in these microbial populations may affect the function of the entire culture.

IMPACT/TECH TRANSFER B: Identification of the microorganisms in the competitive exclusion cultures is necessary to obtain FDA approval and for the ultimate commercialization of this technology. Application of molecular methods may show how the numbers of particular microbes in the population fluctuate under different conditions, affecting the growth of the other microbes, including pathogens. Collaboration has been established with Dr. Rod Mackie, University of Illinois, to further these genetic methods.

OBJECTIVE C: Perform an epidemiological study on the prevalence of *Salmonella* and *Campylobacter* in commercial swine.

PROGRESS C: *Salmonella* survey - Established prevalence of *Salmonella* in market age swine at slaughter from Texas Dept. Criminal Justice (TDCJ). A total of 650 swine from 4 different farrow-to-finish farms (replicated 3 times) were sampled over a 9-month period. We isolated *Salmonella* in 61% of pigs (range 11-88%) with 30+ serovars identified. Fifty-one pigs had more than 1 serovar. Antibiotic sensitivity profiles (13 antibiotics each) have been performed on 362 *Salmonella* isolates from this survey. Two commercially available *Salmonella* ELISA test kits have been evaluated for field studies/HACCP application.

Campylobacter survey - Performed simultaneously with *Salmonella* study, using samples collected from the cecal contents of pigs. The prevalence was 92% (range of 70-100%). *C. coli* was identified in 60% of isolates whereas 31% were *C. jejuni*. Additional studies indicated that pigs were exposed to *Campylobacter* soon after birth and were colonized by 24-48 hrs of age. A repository of *Campylobacter* isolates is being developed and antibiotic sensitivities scheduled.

IMPACT/TECH TRANSFER C: These studies of pathogen incidence will impact the swine industry through establishment of base-line data. Furthermore, these studies form the basis for identifying, along with our cooperator, farms to test new intervention strategies being developed in the Unit. Manuscripts have been submitted to peer reviewed journals outlining the results of these studies. The results of this research were presented at the following scientific conferences: Discover

Conference, Brown State Park, IN; Am. Dairy Sci. Assoc./Am. Soc. Anim. Sci. Annu. Meet., Denver, CO; Rushmore Conf., Mt. Rushmore, SD; Am Assoc. Vet. Lab. Diagn. Annu. Meet., Minneapolis, MN; *Salmonella* Committee, U.S. Animal Health Assoc., Minneapolis, MN; Pork Quality and Safety Summit, Des Moines, IA.

OBJECTIVE D: Develop a swine surgical model to study *in vivo* dynamics of cecal environment and populations of microflora, particularly *Campylobacter*, following feed withdrawal.

PROGRESS D: A swine surgical model was developed to study the effects of standard management practices on microbial integrity of pigs. Studies showed that feed withdrawal, associated with transportation and slaughter operations, can dramatically alter cecal environment and increase *Campylobacter* numbers. In contrast, our preliminary studies suggest that transportation may have a lesser effect on microbial burdens.

IMPACT/TRANSFER D: This is important because of the implications for food safety issues. This research was recognized for its potential impact to the swine industry and was funded in part by a grant from the National Pork Producer's Council. The results of these studies have been submitted for publication and results were presented at the following scientific conferences: Am. Dairy Sci. Assoc./Am. Soc. Anim. Sci. Annu. Meet., Denver, CO; Rushmore Conference, Mt. Rushmore, SD; Am. Swine Practitioners Assoc. Annu. Meet., St. Louis, MO.

OBJECTIVE E: Determine the effect of antibiotics on the gut microflora of swine and how they impact the ability of the normal flora to protect the host animal from pathogen colonization and to determine the potential for acquisition of antibiotic resistance among microbial populations.

PROGRESS E: Models of gastrointestinal communities from antibiotic and non-antibiotic fed swine have been developed via continuous flow culture methodology. These models have been used to assess the impact of antibiotics on populations of commensal and mutualist bacteria and the ability of these populations to resist colonization by antibiotic-resistant and -nonresistant enteropathogens. Moreover, molecular methods are now being applied to assess the effects of antibiotics on certain functional groups of bacteria within the population as well as on the genetic diversity within the populations as a whole. In certain instances, monoclonal antibodies raised against specific bacteria have been used to assess fluctuations in populations and specific interactions occurring between these microbes and drug-resistant salmonellae following periods of antibiotic feeding. Results from these experiments have been presented at national meetings and manuscripts have been submitted for publication in scientific journals.

IMPACT/TECH TRANSFER E: Our development of *in vitro* models of gastrointestinal habitats greatly facilitates research on the impact of antibiotics on normal gut flora and pathogens. Moreover, these models provide for quantitative risk assessments regarding the potential for acquisition of resistance among certain microbial populations. Information gained from these studies has many uses, e.g., such information will aid in establishing guidelines and recommendations regarding types and levels of specific antibiotics used in animal agriculture. Scientists in this CRIS have assisted FDA in formulating and evaluating their extramural antibiotic resistance program.

OBJECTIVE F: Develop a model to predict the growth and competitiveness of *Escherichia coli* O157:H7 in gastrointestinal habitats and to develop microbial-based intervention strategies to control growth of this pathogen in cattle.

PROGRESS F: Microbial communities from fasted and well fed cattle have been established in continuous flow culture. An *in vitro* model of ruminal fermentation has been developed and validated. This model has been used to study the competitiveness of *E. coli* O157:H7 under conditions which simulate gastrointestinal events occurring during transport and slaughter of cattle. Specific nutrients and environmental parameters are being assessed to model factors which cause complete elimination of *E. coli* O157:H7 from the community and information generated from these studies is being incorporated into an intervention strategy designed to limit growth of the pathogen in cattle prior to slaughter.

IMPACT/TECH TRANSFER F: Specific feedstuffs, production practices and/or regional areas of production have been loosely associated with increased incidences of *E. coli* O157:H7; however, in many instances the evidence is circumstantial at best. Our research provides the ability to experimentally examine these variables via a cost effective, reproducible *in vitro* model as well as the ability to validate our findings *in vivo*. For instance, cattle frequently endure periods of feed withdrawal immediately prior to transport and slaughter and our research should determine the effect of such events on the relative proportion of *E. coli* O157:H7 in microbial populations in the rumen and lower gastrointestinal tract. Moreover, a priority of this research is the development and implementation of practical and affordable intervention strategies that will suppress the growth of *E. coli* O157:H7 in cattle. In this regard, studies are underway to elucidate factors which limit the competitiveness of this pathogen in gastrointestinal habitats. We have presented results of research at national meetings and by invitation to the National Cattleman's Beef Association. Dr. Nisbet's expertise in gastrointestinal microbiology has been called upon to provide factual information concerning competitive exclusion technology to the national news media.

OBJECTIVE G: To produce highly specific monoclonal antibody-based immunoassays capable of detecting enteric pathogens and normal gastrointestinal bacteria in poultry and swine.

PROGRESS G: Antibody reagents have been extensively used in biology and medicine to detect and confirm the presence of pathogens. The speed and simplicity of immunoassays make these assays attractive for large scale screening or in clinical settings where rapid confirmation is necessary. A series of monoclonal antibodies to normal swine and poultry intestinal bacteria have been produced and used to develop rapid assays. These assays have been used to enumerate bacterial levels in both defined competitive exclusion (CE) cultures developed by the Unit and to track the fate of these bacteria in normal versus CE treated animals. In addition, monoclonal antibodies have been produced to pathogenic enteric bacteria including *E. coli* O157:H7, *Salmonella typhimurium* (phase II i antigen), and *Campylobacter* species. The anti-*Campylobacter* monoclonal antibodies fall into 4 groups: anti-*C. jejuni* specific antibodies; anti-*C. coli* antibodies; anti-*C. lari* antibodies and a fourth group of monoclonals that bind equally to these three species. None of the anti-*Campylobacter* antibodies reacted with any of the *Arcobacter*, or *Helicobacter* species tested or with any of the other enteric bacteria tested. Initially, some of these antibodies were used to develop a rapid latex agglutination test for easy typing of *Campylobacter* isolates recovered in the Unit's swine epidemiology study. Studies continue with these highly specific anti-*Campylobacter* monoclonals. Future studies are planned to evaluate these antibodies in novel immunoassay formats with a goal of developing detection schemes that can detect microbes in real-time with no or only very limited tissue culturing requirements. We are evaluating an immunomagnetic electrochemiluminescence sensor (see object E) using these antibodies.

IMPACT/TECH TRANSFER G: The results of our studies enumerating the normal gut bacteria will provide useful information on the mechanisms controlling the effectiveness of competitive exclusion as an intervention strategy for control of pathogens on the farm. Such information will be essential for development of "next-generation" competitive exclusion cultures for pathogen control. The anti-*Campylobacter* antibodies potentially will form a rapid immunochemical method for species typing and confirmation. These antibodies are being evaluated by a commercial kit manufacturer to determine their application in rapid testing schemes. Clearly, real-time immunoassays for pathogens would have direct applications to the food animal industry.

PUBLICATIONS:

Anderson, R.C., D.J. Nisbet, S.A. Buckley, K.J. Genovese, R.B. Harvey, J.R. DeLoach, N.K. Keith and L.H. Stanker. 1998. Experimental and natural infection of newly weaned pigs with *Salmonella choleraesuis*. Res. Vet. Sci. 64:261-262.

Durant, J., C.R. Young, D.J. Nisbet, L.H. Stanker and S.C. Ricke. 1997. Detection and quantification of poultry probiotic bacteria in mixed culture using monoclonal antibodies in an enzyme-linked immunosorbent assay. Intl. J. Food Microbiol. 38:181-189.

Swindle M.M., R.B. Harvey, E. Kasseri and S.A. Buckley. 1998. Chronic cecal cannulation in Yucatan miniature swine. Contemp. Top. Lab. Anim. Sci. 6:35-37.

ABSTRACTS:

Anderson, R.C., D.J. Nisbet, D.E. Corrier and L.H. Stanker. 1997. *In vitro* evaluation of porcine derived competitive exclusion cultures. J. Anim. Sci. 75(Suppl. 1):39.

Anderson, R.C., D.J. Nisbet, S.A. Buckley, K.J. Genovese, R.B. Harvey, C.R. Young, J.R. DeLoach and L.H. Stanker. 1997. Competitive exclusion of the host adapted pathogen, *Salmonella choleraesuis*, from the porcine gut. Biosci. Microflora 16(Suppl.):15.

Anderson, R.C., D.J. Nisbet, S.A. Buckley, J.R. DeLoach and L.H. Stanker. 1997. Evaluation of porcine derived competitive exclusion cultures developed for prevention of infection of swine by salmonellae. Prog. Pig Sci., 58th Easter School Agric. Food Sci., 594.

Anderson, R.C., D.J. Nisbet, S.A. Buckley, R.B. Harvey, K.J. Genovese, N.K. Keith and L.H. Stanker. 1998. Competitive exclusion of *Salmonella* from the pig gut. J. Anim. Sci. (suppl. 1)

Buckley, S.A., D.J. Nisbet, R.C. Anderson and L.H. Stanker. 1998. The effects of nutrient withdrawal on anaerobic and *E. coli* O157:H7 CFU's. J. Anim. Sci. (suppl. 1).

Harvey, R.B., C.R Young, R.C. Anderson, M.M. Swindle, S.A. Buckley and D. J. Nisbet. 1998. Influence of feed withdrawal on cecal environment and *Campylobacter* populations in a swine surgical model. J. Anim. Sci. (suppl. 1).

Hume, M.E., S.A. Buckley, R.L. Ziprin, R.C. Anderson, L.H. Stanker and D.J. Nisbet. 1998. Reduction of *Salmonella typhimurium* in continuous-flow competitive exclusion cultures derived from swine cecal bacteria. J. Anim. Sci. (suppl. 1).

Nisbet, D.J., R.C. Anderson, S.A. Buckley, R.B. Harvey and L.H. Stanker. 1997. Application of a continuous-flow fermentation culture of swine cecal microflora to study interactions between microflora and enteropathogens. Biosci. Microflora 16(suppl. 1):16.

Young, C.R., R.L. Ziprin and D.J. Nisbet. 1998. Isolation and enumeration of *Campylobacter* species from commercial swine operations. J. Anim. Sci. (suppl. 1).

STRATEGIES TO CONTROL SWINE PARASITES AFFECTING FOOD SAFETY

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FSIS NUMBER: B2AA04,05
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OBJECTIVE A: Reduce transmission of foodborne pathogens of swine by defining cytokine-regulated immune mechanisms that protect pigs against parasites that threaten food safety.

PROGRESS A: Disease resistance in neonatal pigs was studied. A detailed analysis of the intestinal immune system defined early development of protective function in the gut. Efforts were aimed at identifying novel biotherapeutics, such as exogenous cytokines, to stimulate immune system maturation in susceptible piglets. Samples from different intestinal tissues were isolated and the lymphocytes purified and effector functions assayed. These studies indicate distinct periods of functional immaturity that presumably contribute to susceptibility to a variety of infectious agents. A porcine IL-12 has been produced that will be used to activate protective responses from neonatal pigs. Irradiated *Toxoplasma gondii* oocysts stimulate protective immunity and decrease clinical disease in pigs, however, protection from a subsequent challenge infection is incomplete. Effective immunity will likely require immune activators. Other studies have demonstrated that *Ascaris suum*, a ubiquitous pig parasite, is infectious for non-human primates and may be a clinically significant human zoonosis. Mice that have a defect in the IL-4 receptor and Stat6-dependent activation of gene expression are more susceptible to a variety of gastrointestinal parasites. There may be a similar mechanism regulating protective responses in man and livestock. The swine cytokines IL12, IL13 and IL15 have been cloned and competitor reagents developed for studying changes in gene transcription. A simple PCR-based test was developed that can differentiate the 7 distinct genotypes of *Trichinella* based upon a variable region within the lsrDNA. Work is in progress to extend this technology to perform analyses at the level of a single parasite.

IMPACT/TECH TRANSFER A: These studies will identify periods of immune system immaturity in neonatal pigs, and evaluate the potential use of immune activators. This information will be valuable for induction of immunity against a variety of infectious diseases in developing pigs. Evaluation of a recombinant porcine IL-12 in neonatal pigs will provide the first information on the

use of immune stimulates in the regulation of infectious diseases in pigs. Development of reagents for studying cytokine gene transcription will assist in understanding the development of immunity in pigs. Using the PCR diagnostic techniques, it was shown that *Trichinella* from wild game obtained from arctic regions of Canada cannot be inactivated by freezing as previously recommended, and also demonstrated that *T. pseudospiralis* is likely the ancestral species of this important genus.

PUBLICATIONS:

Dubey, J.P., J.K. Lunney, S.K. Shen and O.C.H. Kwok. 1998. Immunity to toxoplasmosis in pigs fed irradiated *Toxoplasma gondii* oocysts to pigs J. Parasitol. 84: 749-752.

Lunney, J.K. 1998. Cytokines orchestrating the immune response. OIE Sci. Techn. Rev. 17: 84-94.

Noben-Trauth, N., L. Shultz, F. Brombacher, J. Urban, H. Gu and W. Paul. 1997. An interleukin 4-independent pathway for CD4+ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. P.N.A.S. (USA) 94:10838-10843.

Rhoads, M., R. Fetterer and J.F. Urban. 1998. Effect of protease class specific inhibitor on *in vitro* development of L3 to L4 of *Ascaris suum*. J. Parasitol. 84: 686-690.

Urban, J., N. Noben-Trauth, D. Donaldson, K. Madden, S. Morris, M.F. Collins and F. Finkelman. 1998. Both IL-4 and IL-13 mediate the expulsion of a gastrointestinal nematode parasite through an IL-4 receptor alpha chain and Stat6-dependent mechanism. Immunity 8: 255-264.

IDENTIFICATION AND MAPPING OF GENES INVOLVED IN PARASITIC DISEASE RESISTANCE/SUSCEPTIBILITY

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OBJECTIVE: Identify and breed livestock and poultry that are genetically resistant to parasite infections.

PROGRESS: Genetically defined minipigs, with known swine leukocyte antigen (SLA) haplotypes, were tested to identify genes and factors which help encode genetic resistance to *Toxoplasma gondii* infections. Pigs of different SLA haplotypes were tested for natural resistance to low level infections. Tissue samples from each group of pigs were saved for cytokine expression to define immune mechanisms controlling this resistance. Studies showed a correlation between increases in expression of the cytokine interferon-gamma (IFNg) and enhanced resistance to *T. gondii* infection. Moreover, pigs of the swine SLA d haplotype were more resistant to infection and had fewer cysts recovered from their tissues. Work is being continued to determine whether IFNg is the major cytokine involved. Follow-up studies will use a panel of cytokine probes to determine which cytokines are induced by infection, and which are associated with protective immune responses. Data is being collected on additional individuals, so that detailed mapping of resistance genes can be performed.

IMPACT/TECH TRANSFER: Once genetically resistant swine are identified, producers will be able to begin to identify disease resistant pigs in their own breeding stock. Thus producers who have a problem with T. gondii infections in their herds will be able to increase the numbers of pigs with genetic resistance. Coincidentally researchers will be able to use defined herds to probe the complex genetics of resistance and begin to identify the genes involved in resistance. These studies will help breeders reduce costs of drug and vaccine treatments by selecting for parasite-resistant stock. In areas where these parasitic diseases cannot be eliminated, this alternate approach will result in healthier pigs, and will help prevent parasite contamination of pork products.

PUBLICATIONS:

Lunney, J.K., and J.E. Butler. 1998. Immunogenetics, pp. 163-197. *In:* M.F. Rothschild and A. Ruvinsky (eds.), Genetics of the Pig. CAB Int., Wallingford, UK.

Ramsoondar, J.J., E.B. Rucker, J.C. Vasquez, D.S. Gallagher, D.R. Grimm, J.K. Lunney, L.B. Schook and J.A. Piedrahita. 1998. Isolation and genetic characterization of the porcine apolipoprotein E gene. Anim. Genetics. 29:43-47.

EPIDEMIOLOGY AND CONTROL OF *TOXOPLASMA*, *TRICHINELLA* AND RELATED PARASITES IN DOMESTIC ANIMALS

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OBJECTIVE A: To develop tools necessary for implementation of a pre-harvest certification program for trichinae infection in pigs.

PROGRESS A: Pilot studies, conducted both on-farm and in packing plants in the northeastern United States and Iowa to identify risk factors and to develop methods to monitor good management practices (GMP's), were completed in 1996 and 1997. A farm audit, designed to identify the presence or absence of risk factors for trichinae infection, continues to be tested in Iowa to determine any effects of seasonal variation. Improvements in the audit vehicle to increase objectivity have been made, particularly with respect to rodent control procedures. A final step in validation of the certification system is underway, to assure that trichinae-positive farms are excluded based on deviation from established GMP's. Swine serum samples are being tested from farms in Michigan, Pennsylvania, New Jersey, Connecticut, Massachusetts, Rhode Island, Vermont and New Hampshire. When a group (20-25) of positive farms has been identified and confirmed, audits will be conducted on these farms along with a cohort group of trichinae-negative farms. APHIS and ARS are cooperating on an economic analysis of trichinae control, comparing the costs of testing individual carcasses using the pooled sample digestion method with certification-based on-farm audits and in-plant monitoring.

IMPACT/TECH TRANSFER A: The results of these studies will support certification as a method for assuring the safety of pork with respect to trichinae. Certification for trichinae will serve as a model for programs for other zoonotic diseases.

OBJECTIVE B: Provide training and quality control in a program for the inspection of pork and horsemeat for export to the European Union and Russia; to provide program support in the form of evaluating and improving inspection methods for trichinae in pork and horsemeat.

PROGRESS B: The ARS administers a training and quality control program which includes 9 pork packers, 4 horse packers and 3 feral swine packers which ship certified trichinae-free products to Europe and Russia. Four training sessions are held each year for personnel at participating plants to become certified. On a quarterly basis, check samples are prepared and distributed to all certified trichinae analysts. Accurate analysis of check samples allows continued certification of these inspectors. Research projects to determine the effectiveness of digestion and serology methods for the detection of trichinellosis, primarily in pigs, continue to be performed. This research is used in making modifications to the existing program and is conducted in cooperations with scientists in Canada, Europe and Russia. In FY98, experiments were conducted to determine optimal conditions for performance of pooled sample digestion methods. These studies were initiated due to discrepancies which exist in established methods used in Europe and Russia. The results of these studies will be used to support the methods used by U.S. packers.

IMPACT/TECH TRANSFER B: The results of this program and related research is the assurance of market access for fresh pork products in Europe and Russia.

OBJECTIVE C: Determine the survival of *Toxoplasma gondii* oocysts under defined temperatures.

PROGRESS C: The survival of sporulated *Toxoplasma gondii* oocysts in water at -10 C to 70 C for various periods was investigated. Infectivity of *T. gondii* was tested by bioassay in mice. There was no marked loss of infectivity of oocysts stored at 10 C, 15 C, 20 C, and 25 C for 200 days, whereas there was a 100-fold loss of infectivity of oocysts stored at 30 C for 107 days. Oocysts stored at 35 C were infective for 32 days but not 62 days, at 40 C oocysts were infective for 9 days but not 28 days, at 45 C oocysts were infective for 1 day but not 2 days, at 50 C oocysts were infective for 1 hr but not 2 hr. At 55 C and 60 C oocysts were rendered non-infective in 2 and 1 min, respectively. Oocysts remained infective up to 54 mo at 4 C and there was no loss of infectivity in oocysts stored for 106 days at -5 C, and -10 C, and for 13 months at 0 C.

IMPACT/TECH TRANSFER C: This information will be useful to FSIS for providing guidelines to decontaminate materials contaminated with oocysts.

OBJECTIVE D: Effect of gamma irradiation on unsporulated and sporulated *Toxoplasma gondii* oocysts.

PROGRESS D: The effect of ^{137}Cs irradiation on unsporulated and sporulated *Toxoplasma gondii* oocysts was investigated as a model system for sterilization of fruit contaminated with other coccidia such as *Cyclospora* or *Cryptosporidium*. Unsporulated oocysts irradiated at ≥0.4 to 0.8 kGy sporulated but were not infective to mice. Sporulated oocysts irradiated at ≥0.4 kGy were able to excyst, and sporozoites were infective but not capable of inducing a viable infection in mice.

Toxoplasma gondii was detected in histologic sections of mice up to 5 days but not at 7 days after feeding oocysts irradiated at 0.5 kGy. Transmission electron microscopy revealed that sporozoites from irradiated oocysts penetrated enterocytes and all cells in the lamina propria except for red blood cells. Sporozoites appeared normal ultrastructurally and formed a typical parasitophorous vacuole containing a well-developed tubulovesicular membrane network. Raspberries inoculated with sporulated *T. gondii* oocysts were rendered innocuous after irradiation at 0.4 kGy. Results indicate that irradiation at 0.5 kGy is effective in "killing" coccidian oocysts on fruits and vegetables.

IMPACT/TECH TRANSFER D: This information will be useful to FSIS and FDA for providing guidelines to decontaminate food and food products contaminated with coccidian oocysts.

OBJECTIVE E: Determine control procedures for toxoplasmosis in swine.

PROGRESS E: A 3-year field trial was conducted on 8 commercial swine farms in Illinois to determine the effectiveness of a feline *Toxoplasma gondii* vaccine in reducing the exposure of swine to *T. gondii*. A vaccine consisting of live bradyzoites of the mutant T-263 strain, capable of preventing oocyst shedding by cats, was used in this study. Each farm was visited 3 times in 1994, 3 times in 1995 and once in 1996. Cats were trapped and inoculated with the T-263 oral vaccine during 1994 and 1995. On each visit, the following samples were collected: blood from pigs, cats, and mice for detection of serum antibodies to *T. gondii*, feces from cats to detect oocysts, and heart and brain tissues from rodents to determine presence of *T. gondii* tissue cysts. The modified agglutination test, with a positive titer set at the 1:25 dilution, was used to determine serum antibodies. At first capture, 72.6% (61/84) of juvenile cats and 32.6% (31/95) of adult cats had no detectable antibodies, indicating no prior exposure to *T. gondii* when they received their first vaccine. Of these first time seronegative cats, 58.1% (18/31) of adult and 45.9% (28/61) of juvenile cats were recaptured and received a second dose of vaccine. Changes in the prevalence of *T. gondii* infection were evaluated from the pre-vaccination (1992/93) to the post-vaccination period (1996). Eleven cats (5%) were detected shedding oocysts between 1994-1996, of which 10 (90.1%) shed during 1994. The last detection of oocyst shedding by cats was during the first farm visit in 1995. There was a significant decrease in *T. gondii* seroprevalence for finishing pigs ($p<0.05$, Wilcoxon Sign Rank test). There was a positive correlation (Spearman's rho =1.0 $p<0.0001$) between the change in prevalence in juvenile cats and the change in prevalence in finishing pigs. Seroprevalence in mice among all farms decreased from 4% in 1992/3 to 0% in 1996. The mean rate of tissue cyst isolation for mice on all farms decreased from 1.1% in 1994 to 0.8% in 1995 and to 0.4% in 1996. The results of this study suggest that the reduced exposure of pigs to *T. gondii* was the result of the administration of the *T. gondii* vaccine to cats.

IMPACT/TECH TRANSFER E: The results of this study will be useful in determining strategies to control toxoplasmosis in pigs.

PUBLICATIONS:

Davies, P.R., M.E. Morrow, S. Patton, H.R. Gamble and J. Deen. 1998. Seroprevalence of *Toxoplasma gondii* and *Trichinella spiralis* in finishing swine raised in different production systems in North Carolina, USA. Prev. Vet. Med. 36:67-76.

Dubey, J.P. *Toxoplasma gondii* oocyst survival under defined temperatures. 1998. J. Parasitol. 84:862-865.

Dubey, J.P., J.K. Lunney, S.K. Shen and O.C.H. Kwok. 1998. Immunity to toxoplasmosis in pigs fed irradiated *Toxoplasma gondii* oocysts. J. Parasitol. 84:749-752.

Dubey, J.P., D.W. Thayer, C.A. Speer and S.K. Shen. 1998. Effect of gamma irradiation on unsporulated and sporulated studies indicate distinct periods of functional immaturity that presumably contribute to susceptibility. Int. J. Parasitol. 28:369-375.

Gamble, H.R., M.B. Solomon and J.B. Long. 1997. Effects of hydrodynamic pressure on the viability of *Trichinella spiralis* in pork. J. Food Protect. 61:637-639.

Gamble, H.R. 1998. Sensitivity of artificial digestion and enzyme immunoassay methods for inspection of trichinellosis in pigs. J. Food Protect. 61:339-343.

Gamble, H.R., and G. Bush. 1998. Seroprevalence of *Trichinella* infection in domestic swine based on the National Animal Health Monitoring System's 1990 and 1995 Swine surveys. Vet. Parasitol. (in press)

CONTROL AND PREVENTION OF *CRYPTOSPORIDIUM PARVUM* INFECTION

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CRIS NUMBER: 3625-32000-006
FSIS CODE: N2AM01
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OBJECTIVE A: Develop methods to prevent or minimize *Cryptosporidium parvum* infection in cattle.

PROGRESS A: Intense, early exposure of calves to *C. parvum* in the highly contaminated environment present on most production facilities has been shown to overwhelm any potential protection induced by the first generation vaccine produced in this lab. We are now working to develop a second generation vaccine that will provide quicker protection and thus be more effective in a production setting. We have obtained genes coding for *C. parvum* protein and immune-stimulating molecules that we will insert into bacterial vectors. These constructs will be tested as oral vaccines in newborn animals for their efficiency in inducing a rapid immune response to *C. parvum*.

IMPACT/TECH TRANSFER A: Our published findings indicate that protection of calves from on-farm contamination with *C. parvum* will require better vaccines and treatments along with improved hygiene and management of calves by producers to reduce early exposure to the parasite. We are continuing to write articles and present seminars aimed at producers and veterinarians outlining ways to reduce the impact of *C. parvum* infection through management strategies. These efforts will reduce the levels of preharvest contamination, and reduce the chance of *C. parvum*-contaminated water and food reaching the consumer. In addition, use of these management strategies will facilitate the effectiveness of the second generation vaccine by minimizing the early exposure of calves to *C. parvum*.

OBJECTIVE B: Develop methods to control contamination of the environment by animals infected with *C. parvum*.

PROGRESS B: We have published information on sources of *C. parvum* in the environment of the newborn calf. We found that on a highly contaminated dairy, the parasite could not be found in the adult cows, nor in the soil of barns and pens. However, parasites were found in scrapings from the walls of wooden pens where *C. parvum*-infected calves were held.

IMPACT/TECH TRANSFER B: These findings indicate that moist, porous surfaces are a likely source of new infections for calves. Thus, we have recommended to producer groups and veterinarians that stalls be cleaned and thoroughly dried after housing infected calves before being used to house newborn, noninfected calves. These efforts will reduce the early exposure of calves to the parasite, thus breaking the cycle of transmission, and further environmental contamination.

PUBLICATIONS:

Atwill, E.R., J.A. Harp, T. Jones, P.W. Jardon, S. Checel and M. Zylstra. 1998. Evaluation of periparturient dairy cows and contact surfaces as a reservoir of *Cryptosporidium parvum* for calfhood infection. Amer. J. Vet. Res. 59:1116-1121.

Harp, J.A. 1997. *Crypto* can be a calf raiser's nightmare. Hoard's Dairyman 142:302.

Harp, J.A., and J. P. Goff. 1998. Strategies for the control of *Cryptosporidium parvum* infection in calves. J. Dairy Sci. 81:289-294.

ABSTRACTS:

Harp, J.A. 1998. What you see is not always what you get: Suggestions for the control of *Cryptosporidium parvum* infection in calves. Proc. Symp. Minnesota Dairy Health Conf., St. Paul, MN, 65-68.

PREVENTION & THERAPY FOR PROTOZOAN PARASITES AFFECTING FOOD ANIMALS, FOOD SAFETY, PUBLIC HEALTH

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OBJECTIVE: Prevent illness in food animals, control food contamination and protect public health from protozoan parasites. *Cryptosporidium parvum* affecting all mammals will be a target of immunity-based strategies.

PROGRESS: Following a series of experiments that demonstrated eastern oysters from the Chesapeake Bay can remove *Cryptosporidium parvum* oocysts from artificial seawater and store them in an infective state in gills and hemocytes for up to 1 month the team found naturally infected oysters in 11 tributaries of the Bay, some associated with possible areas of runoff from farms with cattle. Mussels collected from the same areas were also found to harbor *C. parvum* oocysts.

Based on last year's study of 9-day old calves infected with *Cryptosporidium parvum* in which the infection elevated the interleukin-12 (IL-12) and gamma interferon intraepithelial cells, a study was initiated to determine if exogenous recombinant IL-12 would prevent infection in young calves. The bovine genes were cloned and expressed by ARS scientists, and the recombinant was produced under a CRADA with Schering-Plough Corporation. Despite the use of relatively high levels of IL-12, no protection was observed.

Oocysts of *C. parvum* irradiated at 20 Krads rendered oocysts noninfectious for mice but they remained infectious for neonatal cattle.

IMPACT/TECH TRANSFER: By detecting *Cryptosporidium* in oysters and mussels we have shown that shellfish can be good indicators of fecal pollution of surface waters and that they pose a public health risk if eaten raw. Utilizing PCR testing for detection of *Cryptosporidium* increases our ability to detect low-level infections, differentiate species infectious for humans vs those noninfectious for humans, and, in some cases, to determine the source of the fecal contamination. The finding that exogenous IL-12 alone did not protect calves from experimental infection with *Cryptosporidium*, taken with our earlier findings in mice that IL-12 protected against

cryptosporidiosis, leads us to examine additonal immunomodulators for development of a prophylactic treatment for calves. Because 20 Krads of radiation did not kill oocysts, we will examine the effects of increased radiation doses.

PUBLICATIONS:

Canals, A., P. Pasquali, D.S. Zarelenga, R. Fayer, S. Almeria and L.C. Gasbarre. 1998. Local ileal cytokine responses in cattle during a primary infection with *Cryptosporidium parvum*. J. Parasitol. 84:125-130.

Fayer, R., T.K.Graczyk, E.J. Lewis, J.M. Trout and C.A. Farley. 1998. Survival of infectious *Cryptosporidium parvum* oocysts in seawater and eastern oysters *(Crassostrea virginica)* in the Chesapeake Bay. Appl. Environ. Microbiol. 64:1070-1074.

Fayer, R., T.K. Graczyk, C.A. Farley, E.J. Lewis and J.M. Trout. 1997. The potential role of oysters and waterfowl in the complex epidemiology of *Cryptosporidium parvum*. J. Amer. Water Works Assoc. 15:153-158.

Fayer, R., L.C. Gasbarre, P. Pasquali, A. Canals, S. Almeria and D.S. Zarlenga. 1998. *Cryptosporidum parvum* infection in bovine neonates: Dynamic clinical, parasitic, and immunologic patterns. Int. J. Parasitol. 28:49-56.

Graczyk, T.K., M.R. Cranfield, P. Helmer, R. Fayer and E.F. Bostwisk. 1998. Therapeutic efficacy of hyperimmune bovine colostrum against clinical and subclinical *Cryptosporidium serpentis* infections in captive snakes. Vet. Parasitol. 74:123-132.

Graczyk, T.K., R. Fayer, J.M. Trout, E.J. Lewis, C.A. Farley, I. Sulaiman and A.A. Lal. 1998. *Giardia* sp. cysts and infectious *Cryptosporidium parvum* oocysts in the feces of migratory Canada geese (*Branta canadensis*). Appl. Environ. Microbiol. 64:2736-2738.

Jenkins, M.C., J. Trout and R. Fayer. 1998. Development and application of an improved semiquantitative technique for detecting low-level *Cryptosporidium parvum* infections in mouse tissue using polymerase chain reaction. J. Parasitol. 84:182-186.

Olson, E.J., W.B. Epperson, D.H. Zeman, R. Fayer and M.B. Hildreth. 1998. Effects of an allicin-based product on cryptosporidiosis in neonatal calves. J. Amer. Vet. Med. Assoc. 212:987-990.

Part II. PATHOGEN CONTROL DURING SLAUGHTER AND PROCESSING (Inspection Technology)

CONTROL OF PATHOGENIC AND SPOILAGE BACTERIA ON RED MEAT

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OBJECTIVE A: Visualize bacterial attachment of artificially inoculated beef carcass tissues in real time as a means to study attachment and detachment of bacteria to carcass surfaces.

PROGRESS A: A bioluminescent strain of *E. coli* O157:H7 was genetically engineered that had growth and attachment characteristics similar to the wild type strain (ATCC 43888). This strain (which is non-toxigenic) was inoculated directly to beef carcass surface tissues and monitored by measuring light emission (bioluminescence) using a photon counting intensified CCD camera system and a standard 50 mm photographic lens. Results indicated that the bioluminescent reporter strain could be monitored in real time from the tissue surfaces (ranged in size from 2 x 6 cm to 10 x 10 cm; and could be larger) without any form of sampling. This system allowed the actual real time monitoring of the inoculum on the carcass surface without sampling, plate counting or any delay in time. Spatial and temporal information on the attachment process was not lost as is done through normal destructive sampling techniques.

Attachment and subsequent removal by a simulated water washing procedure was readily observable by the biophotonic method. Results indicated that on intact beef carcass surfaces, the inoculum was more readily removed from the adipose covered tissues than from the lean beef carcass surface tissues. Percent loss of biophotonic signal (light emission) agreed closely with percent loss of viable counts. Tissues taken from diverse sites on the outside portion of the beef carcass indicated that gross attachment does vary according to tissue types but that this variation seems to be a function of tissue topography (smooth versus rough) rather than some specific chemical mechanism. The first

phase of this research is to demonstrate the concept (that using bioluminescent strains, gross contamination could be visualized in real time) has been accomplished and submitted for publication. Since the camera system to conduct this work is located at another institution (the Stanford University Medical School laboratory of Dr. Christopher Contag) any subsequent research using a photon counting macro imaging camera system will rely on continued collaboration between ARS scientists and Stanford researchers, unless such a camera system is obtained in the future.

IMPACT/TECH TRANSFER A: To our knowledge this is the first demonstration of the real time macro-visualization of bacterial attachment to beef carcass surface tissues. Data obtained in this manner will have impact in two ways. First, it will allow us to understand the basis of microbial attachment and detachment to animal carcasses. This technique could potentially allow the monitoring of specific gene expression (such as those sets of genes responsible for bacterial survival on carcasses) *in situ*. Secondly, this technique offers a more rapid means to evaluate antimicrobial carcass treatments that does not rely on sampling, culturing and back-extrapolation of the resulting plate counts to large surface areas. This method is both faster and could be more representative than is the standard plate count for evaluating antimicrobial interventions. The biophotonic technique accounts for large carcass surface areas (our research used 2 x 6 and 10 x 10 cm sizes) without extrapolating sample count data.

OBJECTIVE B: Study attachment of bacterial pathogens to specific carcass surface tissues and component molecules by fluorescent and luminescent genetic reporter strains of *E. coli* O157:H7.

PROGRESS B: This project is in the start-up phase. The reporter strains of *E. coli* O157 have been developed and shown to exhibit phenotypic characteristics that are identical to the parent strain of *E. coli* O157. The fluorescent strain is a transformant of the plasmid pBAD-GFPuv, which fluoresces based on inducible expression of the green fluorescent protein (GFP), and is detectable with our unit's microplate multi-label counter (Wallac Instrument's Victor2; acquired summer 1998). The luminescent strain is a transformant bearing the plasmid pCGLS1 that emits a constitutive signal of light emission detectable in Victor counter. To date we have demonstrated that light emission from inoculated tissues is detectable using the Victor instrument. Also, washing steps can be monitored for decreases in luminescence. We are evaluating this loss of light signal with concomitant plate counts of the reporter strain.The fluorescent labeled *E. coli* will be used in studies to quantitate attachment to various defined substrata including collagen, fatty tissues, and ground beef components. The fluorescent studies will be conducted on tissue components that are bound to membranes and the wells of microtiter plates.

IMPACT/TECH TRANSFER B: This series of experiments will provide data on the contribution of individual carcass tissue components that affect attachment/detachment to the carcass surfaces prior to further processing and even through grinding. This information will be highly useful in designing decontamination interventions for the red meat industries.

OBJECTIVE C: Evaluate combinations of multiple antimicrobial interventions on beef and pork trim for affect on survival of pathogen and the resulting microbial profile of the product.

PROGRESS C: Phase one of this project is the construction of a pilot-plant facility to apply antimicrobials to trim meat. This phase will be accomplished by November of 1998 with the completion of a chamber with belt-driven processing table in the MARC pilot plant building. Phase two will evaluate combinations of interventions that are already FSIS-approved for use in other points of processing. Hot water, lactic acid sprays and hot air-desiccation will be applied alone and in combinations on inoculated beef trim taken through a simulated holding step, ground into 80% lean ground beef, and stored in chubs. Microbial analyses will include total coliforms, *E. coli* biotype 1, mesophilic and psychrotrophic aerobic plate counts, total lactic acid bacteria plate counts and total sporeformer plate counts. Phase three will evaluate the same antimicrobial intervention combinations on the microbial profile of treated, vacuum-packaged, and refrigerated pork loins. Subsequent work will address specific pathogen reduction by the additive effects of several intervention methods.

IMPACT/TECH TRANSFER C: To our knowledge, this pilot-plant trim table facility is the only one of its kind in the research community. Using this facility we will be able to evaluate standard and novel antimicrobial interventions with a high degree of duplication of actual industrial practices.

OBJECTIVE D: To determine if a triterpenoide glycoside, known as saponin, could be used in combination with acetic acid spray washing for carcass decontamination of *E. coli* O157:H7 and *S. typhimurium*.

PROGRESS D: Saponins are approved for use in the food industry as foaming agents. When combined with the current practice of water or organic acid spray treatments, the foaming property of these compounds may provide an added benefit in carcass decontamination. In the first experiment of this study, lean beef carcass surfaces were experimentally inoculated with a fecal slurry containing antibiotic resistant *Escherichia coli* O157:H7 and *Salmonella typhimurium*. Spray washing treatments with saponin-water (SW) or saponin-acid (SA) were more effective for reducing aerobic bacteria than saponin (S), water (W), or 2% acetic acid (AA) washes alone. However, SW and SA treatments were equally or not as effective as AA for reducing populations of *E. coli* O157:H7 or *S. typhimurium*. In the second experiment, experimentally inoculated beef surfaces were

subjected to spray treatments with water-water (WW), water-acid (WA), SW, or SA. When examined against all populations, SW or SA were as effective treatments as WW or WA for reducing aerobic bacteria, *E. coli* O157:H7, and *S. typhimurium* from beef surfaces.

IMPACT/TECH TRANSFER D: The use of saponin in combination with water or 2% acetic acid spray washes was no more effective than combination washes of water with water or 2% acetic acid. Reductions associated with the combination spray treatments may be attributed to the physical removal of bacteria during the spraying process, and not to any specific action of saponin.

OBJECTIVE E: To determine if the compound, cetylpyridinium chloride, could be used as a carcass decontaminating agent for reducing populations of *E. coli* O157:H7 and *S. typhimurium* from lean and adipose beef surfaces immediately after spray washing and over long term, refrigerated vacuum packaged storage.

PROGRESS E: Cetylpyridinium chloride (CPC) is a water soluble, neutral pH, colorless compound that has been used for over 40 years in oral hygiene products including toothpaste, throat lozenges, and mouthwashes. In the first study, a fecal slurry containing antibiotic resistant *E. coli* O157:H7 and *S. typhimurium* was inoculated onto pre-rigor beef shortplates composed of lean surfaces, left untreated, or spray washed (125 psi, 15 s, 35°C) with water or 10 mg/ml CPC. Not only did CPC immediately reduce populations of *E. coli* O157:H7 and *S. typhimurium* to virtually undetectable levels (0 log10 CFU/cm^2), but aerobic plate counts (APC) were also effectively reduced to 0.6 log10 CFU/cm^2. After 35 days of refrigerated (4°C), vacuum-packaged storage, it was demonstrated that CPC also exhibited residual activity such that populations of APC, *E. coli* O157:H7 and *S. typhimurium* were suppressed to levels of 1.70, 0.00, 0.00 log10 CFU/cm^2, respectively. Selective enrichment of Day 35 samples from lean beef surfaces did not recover either of the pathogens. In the second experiment, pieces of adipose tissue taken from pre-rigor beef surfaces were inoculated with a fecal slurry containing the pathogens, left untreated, or spray washed (125 psi, 15 s, 35°C) with water or 10 mg/ml CPC. CPC immediately reduced populations of *E. coli* O157:H7 and *S. typhimurium* on adipose tissue to low levels (< 1.5 log10 CFU/cm^2). Following 35 days of refrigerated, vacuum packaged storage, aerobic and pathogenic bacterial populations demonstrated significant growth on adipose tissues but bacterial populations never reached the levels of untreated controls.

IMPACT/TECH TRANSFER E: This study demonstrates that CPC could be an effective decontaminating agent for reducing both aerobic and pathogenic bacteria on beef surfaces, thereby improving the microbiological safety, stability, and overall quality of beef products.

OBJECTIVE F: To determine if plastic containing 1500 ppm of triclosan could inhibit bacteria in plate overlay assays and bacteria associated with meat surfaces.

PROGRESS F: Triclosan is a nonionic, broad spectrum, antimicrobial agent that has been incorporated into a variety of personal hygiene products, including hand soaps, deodorants, shower gels, mouthwashes, and tooth pastes. In this study, plastic containing 1500 ppm of triclosan was evaluated in plate assays and meat experiments as a means of reducing populations of bacteria. Plate overlay assays indicated that the triclosan-incorporated plastic (TIP) inhibited the following organisms: *Brochothrix thermosphacta* ATCC 11509, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 12598, Bacillus subtilis ATCC 6051, Shigella flexneri ATCC 12022, *Escherichia coli* ATCC 25922, and several strains of *E. coli* O157:H7. In meat experiment 1, irradiated, lean beef surfaces inoculated with *B. thermosphacta*, *S. typhimurium*, *E. coli* O157:H7 or *B. subtilis*, were covered with TIP, vacuum packaged and stored for 24 h at 4°C. Of the organisms tested, only *B. thermosphacta* was slightly inhibited. In meat experiment 2, pre-rigor beef surfaces were inoculated with *E. coli* O157:H7, *S. typhimurium*, or *B. thermosphacta*, incubated at 4°C for 24 h, wrapped in TIP or control plastic, vacuum-packaged, and stored at 4°C for up to 14 days. There was slight inhibition of the organisms after initial application with TIP. However, bacterial populations following long term, refrigerated vacuum packaged storage up to 14 days were not statistically or numerically different than controls. In meat experiment 3, even TIP-wrapped, vacuum packaged, beef samples that were temperature abused at 12°C, did not exhibit significant or sustainable reductions after 14 days of refrigerated storage. Another study indicated that pure cultures of *E. coli* O157:H7 or *B. thermosphacta* added directly to TIP were not inhibited after 2 hours of refrigerated storage or that the antimicrobial activity could be extracted from the plastic.

IMPACT/TECH TRANSFER F: This study demonstrates that while antimicrobial activity is detected against bacterial cultures in antimicrobial plate assays, TIP does not effectively reduce bacterial populations on refrigerated, vacuum packaged meat surfaces.

OBJECTIVE G: Determine the contribution of hide fecal soil to the carcass microbial profile.

PROGRESS G: This series of experiments addresses the issue of whether the hide (especially the perianal and inner thigh areas) are a significant source of bacterial contamination to the finished carcass. Evidence exists that the carcass tag (hide soil) score does not correlate with the final microbial populations of bacteria on finished carcasses. While this concept appears to be valid, we wish to determine if the *E. coli* biotype 1 populations on the carcass are related to those on the hide. Phase one is to collect *E. coli* biotype 1 libraries from the perianal and inner thigh areas and, from the same animal, the carcass with the hide removed pre-split, post split but pre-wash and post-wash. Samples of colonic feces and rumen content will also be evaluated from the same animal followed throughout the actual dressing procedure in a commercial processing plant. In phase two, genetic profiles of the strains will be made through PFGE analysis and, based on that data, ribotyping (RiboPrinterT). An agreement to borrow the RiboPrinterT equipment from the manufacturer is in progress.

IMPACT/TECH TRANSFER G: Should we find that the perianal hide soil is a significant source of carcass microbial contamination, the resulting information could direct greater emphasis on pre-hide removal and pre-evisceration antimicrobial interventions. Such interventions might be altered hide pattern cutting or possibly washes specific for the affected areas of the hide to be performed post stunning but pre-hide removal.

OBJECTIVE H: To determine the immediate and long term effects of organic acid, hot water, or trisodium phosphate spray washes against different strains of enterohemorraghic *E. coli*, *S. typhimurium* DT104, *S. typhimurium*, *Campylobacter coli,* and *C. jejuni* on vacuum packaged, refrigerated beef.

PROGRESS H: In the first phase of this study, a fecal slurry containing a nalidixic acid-resistant strain of *S. typhimurium* and a strain of *S. typhimurium* DT104 were inoculated on to pre-rigor beef shortplates. These surfaces, composed primarily of lean beef carcass tissue, were left untreated or spray washed (125 psi, 15 s, 35°C) with water, 2% lactic or acetic acid, 10% trisodium phosphate, or water followed by hot water (40 psi, 15 s, 65°C). Aerobic and pathogenic bacteria were enumerated immediately after treatments, after 2 days of refrigerated storage, and up to 35 days of 4°C vacuum-packaged storage. The preliminary data indicates that both strains of *S. typhimurium* behave similarly immediately following spray washing and after long term refrigerated storage.

IMPACT/TECH TRANSFER H: The information obtained from this study will determine if spray washing with antimicrobials is effective for reducing enteric pathogens, including multi-antibiotic resistant *S. typhimurium*, *Campylobacter* spp., or enterohemorraghic *E. coli.*

PUBLICATIONS:

Cutter, C.N. 1998. New intervention technologies. Proc. Recip. Meat Conf. 51:133-139.

Cutter, C.N., and G.R. Siragusa. 1998. Incorporation of nisin into a meat binding system to inhibit bacteria on beef surfaces. Lett. Appl. Microbiol. (in press)

Dorsa, W.J., C.N. Cutter and G.R. Siragusa. 1998. Bacterial profile of ground beef made from carcass tissue experimentally contaminated with pathogenic and spoilage bacteria before being washed with hot water, alkaline solution, or organic acid then stored at 4 or 12°C. J. Food Protect. (in press)

Dorsa, W.J., C.N. Cutter and G.R. Siragusa. 1998. Long-term bacterial profile of refrigerated ground beef made from carcass tissue, experimentally contaminated with pathogens and spoilage bacteria after hot water, alkaline, or organic acid washes. J. Food Protect. (in press)

Siragusa, G.R., C.N. Cutter and J.L. Willett. 1998. Incorporation of bacteriocin in plastic retains activity and inhibits surface growth of bacteria on meat. Food Microbiol. (in press)

Waite, B.L., G.R. Siragusa and R.W. Hutkins. 1998. Bacteriocin inhibition of two glucose transport systems in *Listeria monocytogenes*. J. Appl. Microbiol. 84:715-721.

DEVELOP ON-LINE VERIFICATION AND INTERVENTION PROCEDURES FOR HACCP IN SLAUGHTER/PROCESSING SYSTEMS

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OBJECTIVE A: Detection of enterohemorrhagic *E. coli* (EHEC) virulence factor genes in bovine fecal samples that are *E. coli* O157:H7 and EHEC negative. The specific experimental objective is to test the hypothesis that known pathogenicity genes (virulence factors) of EHEC are distributed amongst other non-EHEC intestinal microflora.

PROGRESS A: We have validated PCR procedures to detect virulence factors from *E. coli* O157 (Stx1, Stx2a,2b, eae, hly, chuA) from fecal cultures enriched for total enterobacteriaceae. To date, we have not found any samples (from animals in small feedlot type holding pens) that were culture negative for *E. coli* O157 but also PCR positive for any of the above listed gene products. The next phase of the study is to begin sampling cattle from the large MARC feedlot.

IMPACT/TECH TRANSFER A: This research could provide evidence that the ultimate reservoir, if not source, of virulence factor genes from the enterohemorrhagic *E. coli* is not only other *E. coli* but perhaps non-*E. coli* enterobacteriaceae. Such biological information might help in development of means to lower the fecal shedding rates of this pathogen that present themselves as post-harvest contaminants.

OBJECTIVE B: Develop rapid means to concentrate bacteria from beef carcass and ground beef samples to enhance pathogen detection limits.

PROGRESS B: Non-cultural enrichment methods were utilized to rapidly remove and concentrate bacteria from meat sample suspensions. Experimentation revealed that hydroxyapatite crystals (HA) had a very high binding affinity for a number of bacterial pathogens including *Salmonella* spp., *E. coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Yersinia enterocolitica*, and that maximum cell adherence took place within five minutes. Bacteria in buffer suspensions, bovine carcass sponge samples, ground beef suspensions, and bovine fecal samples were bound and

removed from suspension by HA at levels as high as 99% of the original population. HA concentration of bacteria from ground beef and bovine carcass sponge samples was examined as a method to enhance and speed PCR (polymerase chain reaction) detection of low levels of *Salmonella typhimurium* in these samples. Retail ground beef and sponge samples taken from 24 h chilled carcasses were inoculated with progressively lower levels of *S. typhimurium* such that prepared sample stomachates contained levels of *S. typhimurium* from 10^0 to 10^5 cells/ml. Concentrated (10% HA) and unconcentrated samples were prepared for PCR after 0, 2, 3, or 4 h of nonselective enrichment. Percent adherence values of *Salmonella* from the ground beef and sponge stomachates ranged from 81.3 to 99.4%, and typically were greater than 95% regardless of the sample type or the initial level of *Salmonella*. Without HA concentration and enrichment, *Salmonella* in ground beef was not detected by seminested PCR and agarose gel electrophoresis, even when present at levels up to 10^5 cells/ml in the 1:10 stomached samples. However, when ground beef samples were extracted with HA, limits of detection in non-enriched samples were 10^2-10^3 CFU/ml and in enriched samples were 10 CFU/ml (after 2 and 3 h enrichment) and 1 CFU/ml (after 4 h enrichment). Without concentration or enrichment, the limit of detection of *Salmonella* in bovine carcass sponge samples was 10^3 cells/ml. HA concentration of these non-enriched sponge samples lowered this limit to 100-101 CFU/ml. These findings demonstrate the utility of HA concentration to enhance and speed PCR detection of low levels of *Salmonella* in ground beef and carcass sponge samples.

IMPACT/TECH TRANSFER B: The potential impact of this concentration method is as a means to shorten assay time for samples that normally require several hours of cultural enrichment incubation. This would include carcass surface samples and ground beef, but could potentially be used for other species samples including poultry or pork. In addition to shortening the time required to obtain results, efficient methods of bacterial concentration would allow for the collection of larger, more representative samples, thereby increasing the probability of detection. Because bacteria adherence to HA is nonspecific, this method may easily be adapted to existing PCR protocols for other pathogens. Other rapid test formats including ELISA or culture-based methodologies potentially could benefit by this concentration protocol. This information has been disseminated via presentations made to professional and industrial groups.

OBJECTIVE C: Determination of the induction of genes involved in the development of acid resistance by *Escherichia coli* O157:H7 upon organic acid spray washing of beef.

PROGRESS C: Sampling, RNA isolation, and RT-PCR (reverse transcription-polymerase chain reaction) procedures for studying gene expression of bacteria on beef carcass surfaces were developed. *E. coli* O157:H7 isolates that exhibit inducible acid tolerance to acetic and lactic acids were identified for use in these studies. Candidate genes involved in the development of acid tolerance by *E. coli* O157:H7 were identified. Primers for these selected genes were used to confirm the presence and amplification of the genes in the test isolates. *In vitro* experiments utilizing broth

media with and without acetic and lactic acids were performed using the test isolates, and total RNA has been isolated. This RNA will be used in RT-PCR, and to confirm induction of the chosen acid-shock genes. When candidate acid-shock genes have been confirmed, the *in vivo* studies will be initiated, to determine whether or not these genes are induced upon organic acid spray washing of beef carcass surfaces.

IMPACT/TECH TRANSFER C: It is important to confirm that organic acid spray wash interventions increase the safety and quality of meat, and do not result in the development of acid tolerance or in cross-protective bacterial resistance to further processing or preservation treatments, or contribute to the development of increased virulence. This information will be disseminated via presentations made to professional and industrial groups and by publication in a professional journal.

OBJECTIVE D: Determine the effects of differences in acid resistances and induction of acid tolerances of *E. coli* O157:H7 on the efficiency of organic acid spray washing of beef carcasses.

PROGRESS D: The relative acid resistances and the ability to induce acid tolerance was determined for a collection of *E. coli* O157:H7 strains, by testing survival of acid-adapted and non-acid-adapted cells in HCl (pH 2.5), and in varying percentages of lactic acid and acetic acid. These results indicate that different *E. coli* O157:H7 strains exhibit a variety of acid resistance/tolerance characteristics, ranging from pH-independent acid resistance to relative acid sensitivity. For many strains, the induction of tolerance to low pH (pH 2.5; HCl) and to organic acids was demonstrated. *E. coli* O157:H7 strains that are acid resistant, acid sensitive, or that demonstrated inducible acid tolerance were selected from among the screened collection for further studies to determine if these differences in acid-resistance characteristics can play a role in the efficacy of organic acid spray washing to reduce microorganisms on beef carcass surfaces. *E. coli* O157:H7 was cultivated for 18 h in tryptic soy broth with and without 1% glucose to produce acid-adapted (A) and non-acid-adapted (NA) cells. Beef carcass surface tissue was inoculated with bovine feces containing 10^6 CFU/ml of A or NA *E. coli* O157:H7. The tissue was then subjected to either a water spray wash or a 2% (vol/vol) acetic acid spray wash (both washes at 35°C, 125 psi, 15 s, with oscillation rate of 60/min). A control group was left untreated. After treatments, tissue was refrigerated at 5°C for 2 d, then vacuum-packaged and held at 5°C for a total of 14 d. Preliminary results indicate that differences in acid resistances and/or the induction of an acid tolerance response may affect both initial reductions of *E. coli* O157:H7 and populations at 14 d, but more experimentation is necessary.

IMPACT/TECH TRANSFER D: The use of organic acid spray washes as antimicrobial treatments for carcasses is in use by many segments of the beef processing industry. Feces are a common vehicle for the microbial contamination of carcasses. *E. coli* in feces, because of low pH in the rumen and the presence of organic acids in the gastrointestinal tract, may exist in a state of

induced acid tolerance. It is important to confirm that organic acid spray washes are efficacious for the most resistant strains of this bacterial pathogen and for those *E. coli* O157:H7 exhibiting maximal acid tolerance. This information will be disseminated via presentations made to professional and industrial groups and by publication in a professional journal.

OBJECTIVE E: Determine genes and gene products unique to *E. coli* O157:H7.

PROGRESS E: This study will begin with other closely related strains (O55, O11 and O26) that will be used for a subtractive library approach to identifying gene products that are unique to this enterohemorrhagic bacterium. This study has just begun and genomic DNA samples are being prepared for subtractive hybridization.

IMPACT/TECH TRANSFER E: Real-time methods for detection of *E. coli* O157:H7 are needed to enforce zero tolerance. The genes and gene products identified in this study should facilitate development of rapid, sensitive, specific methods for detection of *E. coli* O157:H7 on meat.

OBJECTIVE F: Determine the level of virulent gene expression in *E. coli* O157:H7 usingquantitative PCR.

PROGRESS F: The level of expression of slt-I, slt-II, hlyA, chuA, and eaeA in *E. coli* O157:H7 (ATCC 35150) is to be measured by quantitative PCR. The bacteria will be exposed to environmental conditions routinely encountered by potential pathogens during meat processing and storage. Gene specific primers were designed, the PCR-amplified DNA product gel-purified and sequenced in order to confirm its identity. A PCR mimic which contains primer-specific gene sequences separated by non-homologous DNA was constructed for each gene of interest, gel-purified, quantitated spectrophotometrically, and used for competitive PCR analysis. RNA isolated from logarithmically growing broth cultures exposed to different environmental growth conditions will be reverse transcribed into cDNA and used in a quantitative PCR assay. This will allow for the determination of environmental effects on virulent gene expression.

IMPACT/TECH TRANSFER F: Currently, meat processors routinely spray wash carcasses with water and various acids (lactic and acetic). Also, the carcass is exposed to large variations in temperature and moisture during processing and storage. It is impractical to assume that if a pathogen is present that the various treatments currently implemented will be 100% effective in removing all pathogens present. It is therefore imperative to understand what effect these treatments have on virulent gene expression in those bacteria that do survive treatment. The industry will be able to use this information to validate the current intervention strategies, or identify potential problems so that measures can be implemented to correct potential problem areas.

PUBLICATIONS:

Berry, E.D., W.J. Dorsa, G.R. Siragusa and M. Koohmaraie. 1998. Bacterial cross-contamination of meat during liquid nitrogen immersion freezing. J. Food Protect. (in press)

Berry, E.D., and G.R. Siragusa. 1998. Integration of hydroxyapatite concentration of bacteria and seminested PCR to enhance detection of *Salmonella typhimurium* from ground beef and bovine carcass sponge samples. Appl. Environ. Microbiol. (submitted)

Siragusa, G.R., W.J. Dorsa, C.N. Cutter, G.L. Bennett, J.E. Keen and M. Koohmaraie. 1998. The incidence of *Escherichia coli* on beef carcasses and its association with aerobic mesophilicplate count levels during the slaughter process. J. Food Protect. (in press)

REDUCTION OF BIOFILMS RELATED TO BACTERIAL CONTAMINATION AND PATHOGEN LOAD DURING POULTRY PROCESSING

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OBJECTIVE A: To study the formation and composition of biofilms on processing plant surfaces.

PROGRESS A: Studies to describe the formation of biofilms and the importance of pathogens within the biofilms in the processing plant environment were continued. Digital aroma technology was used to detect and assess bacterial contamination and classify bacterial isolates important to poultry processing as potential pathogens. Also, pathogens such as *Salmonella, E.coli, and Campylobacter* were compared with plant isolates by digital aroma technology, SPME, and mass spectroscopy to develop profiles of the microbial populations. Under a CRADA with Perkin-Elmer Corporation, analysis began of microbial populations that occur at different temperatures in specific areas of the poultry processing plant. Profiles of specific compounds will be used to further develop methods and instrumentation for early detection of bacterial spoilage and the presence of pathogens.

IMPACT/TECHNOLOGY TRANSFER A: Identifying the factors that play a role in pathogen attachment and survival is a necessary step toward determining the relative importance to food safety of pathogens found in the poultry processing plant. These technologies have been transferred to the State Extension Service, action agencies, other scientists, and industry through invited lectures, training sessions, publications, and CRADA.

OBJECTIVE B: To develop methods of preventing the formation of or removing biofilms on processing plant surfaces.

PROGRESS B: New methods are being devised to determine the efficacy of materials and chemical treatments to render surfaces in the processing areas more resistant to bacterial contamination and biofilm formation. Evaluation of protocols for testing the resistance of a mixture of organisms found in the whole carcass rinse to a range of disinfectants and sanitizers commonly used in the food industry continued. Physical and electrochemical treatments of stainless steel, including various

methods of sanding, grinding, and polishing, were tested for inhibition of bacterial attachment and biofilms. After treatment, each of the treated surfaces was less susceptible to bacterial attachment than untreated stainless steel. Stainless steel samples that had been electropolished showed significantly fewer bacterial cells and initial biofilm formations than all others tested.

IMPACT/TECHNOLOGY TRANSFER B: This project has provided the national food safety program and the processing industry with new information on bacterial attachment to processing plant surfaces. Finding the least amount of treatment necessary to effectively inhibit biofilms will be economical for the industry and consumers as well as reduce the impact of agriculture on the environment. News articles and news briefs on this work in 1998 were reported in the USDA Agricultural Research Magazine, Poultry Times, Food Chem News, Meat & Poultry Magazine, Food Technology Alert, Science News, Food Focus Consultancy, Chemical Engineering, Roll Out Newsletter, Food Processing, Technical Insights Newsletter, Food & Nutrition Res. Briefs, Poultry Processing Worldwide, and The Scientist.

PUBLICATIONS:

Arnold, J.W. 1998. Development of bacterial biofilms during poultry processing. Poultry Avian Biol. 9:1-9.

Arnold, J.W., and S.D. Senter. 1998. Use of digital aroma technology and SPME, GC-MS to compare volatile compounds produced by bacteria isolated from processed poultry. J. Sci. Food Agric. (in press)

ABSTRACTS:

Arnold, J.W., and S.H. Silvers. 1998. Treatments for stainless steel that reduce bacterial attachment and biofilm formation. Poultry Sci. 77(suppl. 1):60.

Arnold, J.W. 1998. Characterization of bacterial contamination in the poultry processing plant by digital aroma technology. Meet. Southern Poultry Sci. Soc. Atlanta, GA.

Senter, S.D., and J.W. Arnold. 1998. Detection of chicken breast meat bacterial spoilage using photoionization detection, GC-MS analysis, and concomitant microbial analysis. Poultry Sci. 77(suppl. 1):63.

ENGINEERING INNOVATIONS AND MICRO DEVELOPMENTS TO REDUCE CONTAMINATION OF POULTRY AND EQUIPMENT

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OBJECTIVE A: Determine the physiology mechanisms of feather retention force (FRF) during the initial stages of broiler processing.

PROGRESS A: Ante-mortem and post-mortem FRF does not appear to be influenced by the presence or absence of cutaneous innervation. Electrical stunning and stimulation procedures during processing increase FRF for the pectoral and sternal feather tracts, while the femoral tract was not affected. Similarly, when these feather tracts became wet with brine solution during electrical treatment FRF was elevated. However, following immersion scalding, electrical treatment did not have a detectable affect on the picking efficiency of male carcasses. Female carcasses are 20% smaller than male carcasses (at 6 week of age), and following application of electrical stimulation and scalding had poorer picking efficiency, requiring additional picking. Recently installed killing equipment (Stork Gameco) in commercial broiler processing plants is cutting deeper than previously recommended, without concern for spinal cord integrity. Deeper cuts ensure a more rapid and uniform rate of death prior to scalding and thereby reduce the incidence of scalding unconscious but not yet dead broilers (which would be condemned). Our previously reported work demonstrated that post-mortem FRF was unaffected by spinal cord severing immediately following stunning. Our current results suggest that treatments disabling the nervous system ante-mortem may lower FRF indirectly by altering cutaneous metabolism.

IMPACT/TECH TRANSFER A: This work may explain why ante-mortem treatments have consistently been unsuccessful in substantially altering post-mortem FRF. The significant increase in post-mortem FRF following electrical stimulation only affected the defeathering of female carcasses, and the additional picking required would be readily overcome with the multiple banks of pickers used in commercial processing plants.

OBJECTIVE B: Determine the effects of the duration of feed withdrawal on carcass shrink.

PROGRESS B: Experiments contrasted feed withdrawal programs and revealed that the degree of carcass shrink associated with feed withdrawal depended on the time point at which the full-fed controls were processed for comparison. About half of the carcass shrink associated with feed withdrawal is attributable to growth for those broilers that remained on feed. Partitioning of growth from shrink was possible by determining processed carcass weight for full-fed controls at each time interval that feed withdrawal was initiated.

IMPACT/TECH TRANSFER B: Carcass shrink associated with longer feed withdrawal is about half that previously reported, after adjustment for the continued growth of full-fed controls. Therefore, the duration of feed withdrawal prior to processing may be extended to help achieve zero fecal carcass contamination prior to chilling, without substantially lowering carcass weight and yield of broiler chickens.

OBJECTIVE C: To determine the ability of propionic acid and chloride salts to inhibit the growth of *Salmonella typhimurium*.

PROGRESS C: Findings indicated that high concentrations of propionic acid, potassium chloride, or sodium chloride may inhibit the growth of *S. typhimurium in vitro*. However, mixtures of propionic acid and potassium chloride or sodium chloride were more effective in inhibiting the growth of the pathogen than either substance alone. Additionally, these substances did not cause any sublethal injury of the bacteria that would reduce recovery of *S. typhimurium* on selective media used for isolating and identifying the pathogen.

IMPACT/TECH TRANSFER C: *Salmonella* is a major cause of foodborne illness in humans. Findings indicate that mixtures containing propionic acid and sodium chloride or potassium chloride might be useful in inhibiting the growth of *Salmonella* in foods and on food contact surfaces.

OBJECTIVE D: To determine the effect of feed withdrawal on the physical, chemical, and microbial characteristics of broiler crops.

PROGRESS D: Findings indicated that significant decreases in crop weight and significant increases in crop pH occur within 6 hours of feed withdrawal. Additionally, feed withdrawal was associated with significant decreases in the number of lactic acid bacteria associated with the crop, but no significant changes in the number of *Enterobacteriaceae* isolated from the crop were found.

IMPACT/TECH TRANSFER D: Broiler crops can serve as a major source of carcass contamination during poultry processing. The taxonomic family of bacteria called *Enterobacteriaceae* includes some pathogens that cause foodborne illness. These bacteria may colonize the crop, and feed withdrawal may increase the ability of their to survive in the crop. By examining how feed withdrawal enhances the survival of *Enterobacteriaceae*, methods may be developed to reduce the number of harmful bacteria associated with the crops of broilers during poultry processing.

OBJECTIVE E: To determine the effect of feed withdrawal on the physical, chemical, and microbial characteristics of the ceca of broilers.

PROGRESS E: Research focused on the effect of feed withdrawal on the weight, pH, and native microflora of the ceca of broilers. Results indicated that no significant changes in cecal weight or pH occurred after 24 hours of feed withdrawal. Feed withdrawal was associated with significant decreases in the cecal lactic acid bacteria population and significant increases in the cecal *Enterobacteriaceae* population.

IMPACT/TECH TRANSFER E: The cecum is one of the main sites of colonization of poultry by foodborne pathogens. Results indicated that feed withdrawal causes major changes in the physical, chemical, and microbial characteristics of the ceca. An increase in the number of *Enterobacteriaceae* in the ceca of broilers subjected to feed withdrawal may lead to an increase the number of broilers contaminated by these bacteria during processing. Further understanding of the changes that feed withdrawal causes in the ceca of broilers may facilitate the development of methods that can reduce the number of *Enterobacteriaceae* in the ceca of poultry; thereby reducing cross-contamination of poultry carcasses during processing.

OBJECTIVE F: Effects of electrical stimulation during bleed-out on lower GI responses of carcasses and the final meat quality.

PROGRESS F: A poultry processing equipment manufacturer donated stunning and killing equipment to the unit so our research could more closely mimic that of commercial processors. An electrical stimulator was constructed and placed in line with the donated equipment. The construction is ongoing at this time and testing is planned for the first quarter of FY 99.

IMPACT/TECH TRANSFER F: At the present time there are 14 known plants using the electrical stimulation procedures designed by our research unit, including one in Brazil. One local processor is using the stimulation procedures in conjunction with a prototype brushing machine to assist with

their HACCP compliance for zero tolerance fecal contamination as well as *E. coli* reduction guidelines. Experiment design and testing parameters are underway to determine the overall affect of the stimulation and brusher under commercial conditions.

OBJECTIVE G: Determine concentrations of bacteria in a three-tank, counterflow scalder to improve computer modeling of bacterial cross-contamination during poultry scalding.

PROGRESS G: Concentrations of coliforms, *E. coli,* and salmonellae were determined at the midpoint of each tank of a three-tank scalder operating at 133 F with a line speed of 140 carcasses per minute. Coliform and *E. coli* concentrations decreased significantly between the first, second, and third tanks. Salmonellae were detected in every sample from the first and second tanks with a geometric mean MPN of less than 2 salmonellae per ml of water, but were detected only rarely in the third tank. The computer model indicates that this pattern of bacterial concentration is determined by the way that bacteria are washed off the carcasses and not by the counterflow action of the scalder.

IMPACT/TECH TRANSFER G: Sampling of scald tanks is continuing to develop a pattern of how bacteria leave carcasses and become suspended in water during scalding. There appears to be more cross-contamination during removal of feathers from scalded carcasses than during the scalding process itself. Final culmination of this investigation should lead to a better understanding of what really happens in the scalding systems of commercial poultry processors and the scientific information needed to design equipment that could reduce microbial loads in the early stages of processing.

OBJECTIVE H: Determine whether mixed scalding and picking affects carcass microbiology.

PROGRESS H: The effect of defeathering carcasses between the tanks of a multiple-tank scalder was tested last year with the idea that bacterial contamination caused by defeathering equipment might be mitigated by several subsequent dips in hot scald water. Contrary to expectations, bacterial levels on treatment carcasses were not lower than on conventionally scalded and picked control carcasses. Those experiments were repeated using cloacal plugs to prevent the escape of feces during scalding and picking, to isolate the effects of bacterial contamination coming directly from the intestinal tract versus bacteria already on the skin or feathers of carcasses during pilot plant processing. Under these conditions, intermittently scalded and picked New-York-dressed carcasses were not microbiologically different from conventional carcasses in terms of aerobic bacteria, *E. coli* or *Campylobacter,* regardless of whether intestinal bacteria were escaping.

IMPACT/TECH TRANSFER H: A laboratory-scale scald tank cannot duplicate the high bacterial loads of a commercial scald tank, so carcasses in a processing plant would carry greater numbers of bacteria in wet feathers compared to carcasses in these experiments. Plans are to repeat this experiment using fecally contaminated scald water.

OBJECTIVE I: Determine whether bacterial numbers on defeathered broiler carcasses can be reduced by different flooring in transportation cages.

PROGRESS I: Concentrations of aerobic bacteria, coliforms, *E. coli, Campylobacter* and incidence of salmonellae were determined on New-York-dressed carcasses after transporting and holding broilers in cages with either conventional flooring or a raised, mesh flooring that allowed fecal material to fall through so that it was not in contact with birds. Feathered carcasses were much cleaner from wire-floored cages, but rinses of defeathered carcasses were not significantly different for aerobic bacteria, coliforms, *E. coli, Campylobacter,* or incidence of salmonellae compared to carcasses from cages with conventional flooring.

IMPACT/TECH TRANSFER I: Research in this area is continuing.

OBJECTIVE J: Evaluation of spray scalding for subcutaneous temperatures, picking, and skin appearance.

PROGRESS J: Thermocouples were positioned beneath the skin of broiler carcasses in eight separate locations. Standard immersion scalding at 52 or 56.5 C for two min or a proto-type spray scalder at 60, 65, or 70 C for one min were used to monitor subcutaneous temperature during scalding. Immersion scalding resulted in an exponential-type profile with the lower temperature having less deviation for the monitored locations. Among sampling locations, the spray scald temperatures were divergent, and the highest temperatures were recorded when thermocouples were within the spray patterns. As with the immersion scalded carcasses, lower temperatures for the spray scalding demonstrated less deviation among the monitored locations and a closer grouping of the final temperatures. The only spray scald temperature tested where subcutaneous temperatures approached those of the immersion scalded carcasses was at 70 C. Additional carcasses were scalded, picked, and examined for skin appearance and picking efficiency. All carcasses spray scalded for 60 s had a "cooked appearance" when evaluated. When spray scald times were reduced to 30 s, skin appearance improved, but with the exception of the 70 C trial, picking efficiency was poorer.

IMPACT/TECH TRANSFER J: Modifications of the spray scalding and picking system should result in a feasible alternative to immersion scalding if some additional incentives could be realized by poultry processors. Additional research is needed to evaluate the present spray scalder with

immersion scalding to determine if carcass microbiological quality is influenced by higher scald temperatures. Better external and internal microbiological quality of processed carcasses would be sufficient incentive to make spray scalding an economically feasible alternative to immersion scalding.

OBJECTIVE K: Develop and evaluate new evisceration concepts and techniques to reduce crop breakage.

PROGRESS K: Crop breakage during evisceration of commercial broilers results in spillage of the contents and possible contamination and cross-contamination of carcasses. Three pre-evisceration procedures were compared to determine if crop breakage could be reduced. The techniques were: 1) leaving the head on, 2) removing the head between the first and second cervical vertebrae, and 3) severing the spinal column parallel with the shoulders, then stretching and cutting the neck skin without damaging the crop. The head-off and neck-off techniques resulted in significantly higher extraction of intact crops (88 and 97%, respectively) compared to 15% with the head-on technique.

IMPACT/TECH TRANSFER K: Prior research, at other facilities, have shown that the crop and its contents can be contaminated with enteric pathogens. With more crops left intact during evisceration, the experimental pre-evisceration method of neck removal could lead to less spillage of the crop contents and therefore the possibility of less contamination and/or cross contamination. A commercial processing equipment manufacturer has expressed interest in the concept which could lead to future discussions and equipment development.

OBJECTIVE L: Treatment of broiler carcasses with an herbal extract.

PROGRESS L: A commercial herbal extract on a salt carrier was tested in a simulated chiller to determine if it could be used to enhance the microbiological quality of fully processed broiler carcasses. Carcasses were chilled in a 2 % solution of the extract for 30 minutes at 1 C, then rinsed in tap water before microbiological sampling. Additional carcasses received a standard 30-minute chill in ice water at 1 C. The diluent from the whole carcass rinse was evaluated for total aerobes, Coliforms, *Campylobacter*, and *E coli*. Counts for plant run control carcasses (prior to chill) were 3.7, 2.5, 2.1, and 2.0 log 10 cfu/ml for the respective organisms. The standard chilling treatment significantly reduced counts when compared to plant run controls (2.6, 1.4, 0.7, 0.9). The herbal extract treatment significantly reduced counts even further ($P<.01$) to counts of 0.06, 0.04, 0.01, and 0.00 log 10 cfu/ml for total aerobes, coliforms, *Campylobacter*, and *E. coli* respectively. Detectable levels of the monitored organisms were 1 cell per ml for the *E coli*, coliforms, and total counts and 10 cells per ml for the *Campylobacter*. The microbial counts for the carcasses treated with the herbal extract, in reality, would be considered too low to be detected.

IMPACT/TECH TRANSFER L: A trust was initiated with The Bavaria Corporation to test the herbal extract during processing on the microbiological quality of broiler carcasses. The use of the herbal extract could prove to be an extremely effective intervention step in an overall HACCP plan for commercial processors. The delivery of a safer product to the consumer would be the primary impact and concern for the poultry processors. Secondary benefits would be increased exports with the EU banning exports treated with chlorine. This could re-open those markets as well as maintaining the markets of countries that are presently considering joining the EU. Research continues into time, temperature, and concentrations to find the optimum treatment conditions with lowest cost for the processor.

PUBLICATIONS:

Buhr, R.J., J.A. Cason and G.N. Rowland. 1997. Feather retention force in broilers ante-, peri-, and post-mortem as influenced by carcass orientation, angle of extraction, and slaughter method. Poultry Sci. 76:1591-1601.

Buhr, R.J., J.A. Cason and G.N. Rowland. 1997. Feather retention force in broilers ante-, peri-, and post-mortem as influenced by electrical and carbon dioxide stunning. Poultry Sci. 76:1602-6.

Buhr, R.J., J.K. Northcutt, C.E. Lyon and G.N. Rowland. 1998. Influence of feed withdrawal time on broiler viscera shear. Poultry Sci. 77:758-764.

Buhr, R.J., and G.N. Rowland. 1998. Subcutaneous feather tract denervation does not alter feather retention force ante- and post-mortem in broilers. Poultry Sci. (submitted).

Dickens, J.A., R.J. Buhr and J.A. Cason. 1998. Subcutaneous temperature profile, skin appearance, and picking efficiency of immersion and spray scalded broiler carcasses. Poultry Sci. (submitted)

Hinton, A. Jr. 1998. Inhibition of the growth of *Salmonella typhimurium* by propionic Acid and chloride Salts. Food Microbiol (submitted)

Lyon, C.E., B.G. Lyon and J.A. Dickens. 1998. Effects of carcass stimulation, deboning time, and marination on yield, color and texture of broiler breast meat. J. Appl. Poultry. Res. 7:53-60.

ABSTRACTS:

Buhr, R.J., A. Dickens and G.N. Rowland. 1997. Influence of electrical stunning, electrocution, and electrical stimulation on post-mortem feather retention force in broilers. Poultry Sci. 77:79.

Buhr, R.J., and J.K. Northcutt. 1998. Influence of feed withdrawal on broiler slaughter and carcass weight. Poultry Sci. 77:147.

Cason, J.A. , A. Hinton, Jr., and K.D. Ingram. 1998. Salmonellae concentrations in broiler scald water. Int. Symp. Food-borne *Salmonella* in Poultry, Amer. Assoc. Avian Pathol., Baltimore, MD, 78.

Cason, J.A., R.J. Buhr, J.A. Dickens, M. T. Musgrove and N. R. Stern. 1998. Effect of intermittent scalding and defeathering on broiler carcass bacteria. Poultry Sci. 77:150.

Dickens, J.A., M.E. Berrang and N.A. Cox. 1998. The effect of an herbal extract on a salt carrier during a simulated chill on the microbiological quality of broiler carcasses. Poultry Sci. 77:61.

Hinton, A. Jr. 1998. Use of propionic acid and potassium chloride or sodium chloride to inhibit the growth of *Salmonella typhimurium in vitro*. Poultry Sci. 77:149.

Hinton, Jr., A., R.J. Buhr and K.D. Ingram. 1998. Effect of feed withdrawal on bacterial flora, pH and weights of the ceca of chickens. Poultry Sci. 77:24.

Hinton, Jr., A., R.J. Buhr and K.D. Ingram. 1998. Changes in the normal bacterial flora, pH, and weights of the crops of chickens subjected to feed withdrawal. Poultry Sci. 77:95.

Northcutt, J.K., and R.J. Buhr. 1998. Influence of feed withdrawal on slaughter weight and processing yield of broilers. Poultry Sci. 77:152.

Northcutt, J.K., R.J. Buhr and G.N. Rowland. 1998. The effects of the age of a bruise on skin surface appearance and histological characteristics in broilers. Poultry Sci.77:62.

SPECTRAL RADIOMETRY AS AN ON-LINE INSPECTION TOOL

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CRIS NUMBER: 1270-42440-001
FSIS CODE: B6II01
CRIS TERMINATION DATE: January, 2000

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OBJECTIVE: To develop an automated, real-time system for on-line detection of unwholesome poultry carcasses (as defined by the Food Safety Inspection Service) in slaughter plants, and to develop techniques to detect on-line individual diseases, defects, and/or contamination associated with poultry carcasses identified as unwholesome on the processing line.

PROGRESS: The Instrumentation and Sensing Laboratory (ISL) has developed an industrial prototype poultry inspection system which consists of a visible/near-infrared (Vis/NIR) subsystem and a multispectral imaging subsystem. The first phase of testing of the ISL automated poultry inspection system on processing line was made at Tyson Foods, Inc., New Holland, PA. During plant trials, the system worked under the adverse conditions of temperature, humidity, water, and processing substances found in poultry processing plants. Analysis of the data shows that the visible/near-infrared subsystem worked well, and was robust and consistent. The calibrated model had the ability to classify the birds with a 95% accurracy, equivalent to those obtained from off-line trials at the Wampler-Longacre Plant, West Virginia. Multispectral testing was however, incomplete due to lighting limitations. The in-plant lighting was modified and additional tests were conducted on a total of 1500 normal carcasses and 200 abnormal carcasses. Data collected from these experiment are currently being analyzed.

In order to develop techniques to identify chicken diseases by on-line poultry viscera machine vision inspection, a portable color vision system was designed and assembled. The portable vision system consisted of a CCD color camera, a lighting chamber equipped with a bundled fiber optic ring light, and a frame grabber board installed in a computer. Over 320 samples (livers and hearts) were collected by FSIS veterinarians, representing four poultry postmortem classes, airsacculitis, cadaver, normal, and septicemia. These samples were examined in color spectral regions by a portable color vision instrument. The color feature in the RGB (Red, Green, and Blue) format was well suited for distinguishing the color difference between normal livers from airsacculitis, and cadaver livers. The morphological feature (i.e., the ratio of heart's fat band area to total heart area) was useful to differentiate between normal and septicemia. Neural networks and fuzzy logic paradigms were used

to design classification models (neuro-fuzzy classifier) to identify various combinations of poultry postmortem classes. Preliminary results showed that, for a two-class model accuracy, defined as the proportion of correctly identified livers of a known class, ranged from 95% for normal vs. airsacculitis livers to 87.5% for normal vs. cadaver livers.

Research is also being conducted to apply hyperspectral imaging techniques for assessing safety and quality of poultry carcasses. Hyperspectra-imaging spectroscopy is a relatively new technology that possesses the essential features of both imaging and spectroscopy technologies, and, therefore, has great potential in evaluating food safety and quality. A laboratory instrumentation system has been built, and system calibration and testing has been completed successfully. A computer algorithm has been developed to compose hyperspectral images from line scan images acquired from a spectrometer coupled with a high precision CCD camera. Wholesome chickens and different classes of unwholesome chickens, including septicemia, cadaver, tumor, air-sacculitis, fecal contamination were imaged. Hyperspectral images are being analyzed to develop optimal systems for online inspection of poultry carcasses.

IMPACT/TECH TRANSFER: The research conducted by ISL has been featured in various publications during the past year, for example: Agricultural Research, May 1998; Foodonline.com, June 1998; Meat and Poultry, June 1998; Vision Systems Design, August; Resource, an official publication of the Society for Engineering in Agricultural, Food, and Biological Systems (ASAE), August 1998. In March 1998, ISL researchers discussed the automated poultry inspection system with managers, FSIS veterinarians and inspectors, a representative of the union of the FSIS inspectors, and key workers at Tyson Foods, Inc., New Holland, Pennsylvania. In June 1998, ISL researchers presented a poster and demonstrated ISL research results at the BARC Field Day. A CRADA between ISL and Stork Gamco, Gainesville, GA is under negotiation.

PUBLICATIONS:

Chen, Y.R., R.W. Huffman, M. Nguyen and B. Park. 1998. Classification of on-line poultry carcasses with backpropagation neural networks. J. Food Process Eng. 21:33-48.

Chen, Y.R., M. Nguyen and B. Park. 1998. Neural network with principal component analysis for poultry carcass classification. J. Food Process Eng. (in press)

Park, B., Y.R. Chen and M. Nguyen. 1998. Multispectral image analysis using neural network algorithm for inspection of poultry carcasses. J. Agric. Engin. Res. 69:351-363.

Park, B., Y.R. Chen and M. Nguyen. 1997. On-line identification of unwholesome poultry carcasses by machine vision, vol. 3, p. 1543-1548. *In:* Y. Sawaragi and S. Sagara (eds.), Proc. Int. Fed. Auto Control Symp. Sys. Iden., Fukuoka, Japan.

ABSTRACTS:

Chao, K., Y.R. Chen, B. Park and H. Early. 1998. Using fuzzy inference systems for assessment of color imaging on chicken livers. ASAE Paper No. 983021. ASAE St. Joseph, MI.

Chen, Y.R., and W.R. Hruschka. 1998. On-line trials of a chicken carcass inspection system using visible-infrared reflectance. ASAE Paper No. 983047. ASAE, St. Joseph, MI.

Park, B., and Y.R. Chen. 1998. Real-time multispectral image processing for poultry inspection. ASAE paper No. 983070. ASAE St. Joseph, MI.

CONTROL OF PATHOGENS ON THE SURFACES OF POULTRY

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CRIS NUMBER: 5325-42000-021
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CRIS TERMINATION DATE: August, 1999

OBJECTIVE A: Using chemical and physical techniques of surface modeling identify and describe mechanisms of the attachment of pathogens to surfaces of poultry.

PROGRESS A: None

OBJECTIVE B: Using chemical and physical techniques, evaluate the effect of inhibitors and disinfectants on food product surfaces.

PROGRESS B: Many different bacteria exploit a cell-cell communication device to regulate transcription of multiple target genes in concert with cell density. This communication device, termed quorum sensing, depends on the production of one or more diffusible signal molecules termed autoinducers which enable a bacterium to monitor its own population density. Many gram-negative bacterial species produce N-acylated homoserine lactones. These compounds serve as intercellular signals that facilitate quorum sensing. In order to facilitate investigation of whether such compounds are significant for *Campylobacter jejuni* colonization and virulence, the majority of the previously identified quorum sensing compounds, various serine lactones, have been synthesized. The synthetic compounds will be used in testing reporter strains and in developing analytical procedures.

IMPACT/TECH TRANSFER B: Use of quorum sensing molecules or synthetic analogs of the known compounds may provide a novel approach to inhibiting biofilm formation on food surfaces, and may contribute to basic understanding of how biofilm formation occurs.

OBJECTIVE C: Develop rapid methods for identifying pathogenic bacteria using laser and thermal desorption mass spectrometry and artificial neural network analysis.

PROGRESS C: Rapid analysis of proteins in pathogenic strains of *Campylobacter* can be obtained by a proteomic method called matrix-assisted laser desorption mass spectrometry, or MALDI. In the method, a single bacterial colony is scraped from a culture plate, vortexed briefly to disperse it, and then mixed with a suitable UV-absorbing matrix material. The mass profiles of protein components of the bacteria are obtained. The MALDI spectra contain distinguishing biomarker ions which characterize the pathogenic strains of *Campylobacter* at the species level plus additional MALDI peaks which are common to several *Campylobacter* species. MALDI spectra provide distinctive spectra for *C. jejuni*, *C. jejuni* sub. *doylei C. coli* and *C. fecalis* based on proteins in the 4000 to 20,000 molecular weight range. Additional species of the fourteen members of the *Campylobacter* genus are being measured to evaluate the potential of the method.

A double-blind analysis of 21 *C. jejuni* and *C. coli* correctly classified 20 of 21 strains based on comparison with hippuricase assay, PCR and monoclonal antibody assignments. One of the possible advantages of the MALDI technique is its ability to resolve ambiguous assignments from the hippuricase assay for *C. coli.* A beginning level of sample automation was achieved for the MALDI measurements. Sample targets containing up to 49 samples of bacteria can be analyzed in the MALDI instrument unattended. Several approaches to automatic sample classification are being considered, including principle component analysis, neural network analysis, and a probability-based spectral matching technique previously used for compound identification by mass spectrometry. Additional experiments are underway to analyze *E. coli* O157:H7 and *Salmonella* by the MALDI technique.

IMPACT/TECH TRANSFER C: Mass spectrometric measurements have the potential to be widely used for confirmatory analysis of pathogenic bacteria.

PUBLICATIONS:

Brandon, D.L., K.P. Holland, J.P. Dreas and A.C. Henry. 1998. Rapid screening for benzimidazoles residues in bovine liver. J. Agric. Food Chem. 3653-3656

Flounders, A. W., D.L. Brandon and A.H. Bates. 1997. Patterning of immobilized antibody layers via photolithography and oxygen plasma exposure. Biosensors Bioelectron. 12:447-456.

ABSTRACTS:

Haddon, W.F., G. Full, R.E. Mandrell, M.R. Wachtel, A.H. Bates and L.A. Harden. 1998 Bacterial protein profiling for *Campylobacter* using MALDI-TOF mass spectrometry. Proc. Conf. Mass Spectrometry Allied Topics, Orlando, FL. (in press)

Haddon, W.F., L.A. Harden and R.G. Binder. 1998. Quantification of a Bacterial Mutagen in Simulated Poultry Processing Water. Proc. Conf. Mass Spectrometry Allied Topics, Orlando FL. (in press)

ADHESION OF HUMAN PATHOGENS TO SURFACES OF POULTRY

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OBJECTIVE A: To identify and describe the molecular mechanisms of the attachment of pathogens to surfaces of poultry.

PROGRESS A: Additional strains of *Campylobacter*, *Salmonella*, *Arcobacter* and *E. coli* O157:H7 have been acquired or isolated. Mouse monoclonal antibodies (MAbs) that bind specifically to *C. jejuni* and *C. coli* have been produced and the molecules they bind have been characterized. The antibodies are being used to develop better isolation and detection methods and for research studies of the *in situ* biology and identification of *C. jejuni* by immunohistochemistry, and measurement of attachment of *C. jejuni* to poultry tissues. Confocal microscopy studies of pathogens located on and in foods are ongoing.

IMPACT/TECH TRANSFER A: A provisional patent related to anti-*Campylobacter* MAbs has been filed. Seven companies are evaluating the MAbs for possible licensing for development of detection assays for veterinary, clinical and food inspection use.

OBJECTIVE B: To identify new technology, including new compounds, that can minimize pathogens in foods.

PROGRESS B: Studies of attachment of *Campylobacter* to poultry are ongoing. Initial approaches include fluorescence microscopy, thin layer chromatography and SDS-PAGE bacterial overlay assays, microwell assays of tissue extracts, and chemical analysis of adherence factors.

IMPACT/TECH TRANSFER B: Information on the attachment of pathogens in foods (e.g., single organism, biofilms, exterior/interior surface, etc.), and mechanism of pathogen binding to food surfaces will help producers develop and test strategies to control the amount of viable pathogen contamination and inhibitors of attachment, and/or compounds for controlling the amount of viable pathogen contamination.

ABSTRACTS:

Mandrell, R.E., A.H. Bates, M.R. Wachtel and D.L. Brandon. 1998. Production of monoclonal antibodies for analysis of *Campylobacter jejuni* and *C. coli* attachment to poultry surfaces. Annu. Amer. Soc. Microbiol., Atlanta, GA, P-50.

ADVANCED TECHNOLOGIES FOR REDUCTION OF MICROORGANISMS AND PARTICULATE MATTER IN FOOD PROCESSING

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OBJECTIVE A: Develop safe and efficient methods for disinfecting food processing waters, including methods for analyzing disinfectant chemicals.

PROGRESS A: An instrument that determines chlorine dioxide accurately in the presence of chlorine and/or other oxidants has been developed. The instrument gives instantaneous analytical results. It will be useful for monitoring chlorine dioxide residuals in the disinfection of processing waters as a means of insuring adequate residual levels and to minimize unnecessary overuse.

IMPACT/TECH TRANSFER A: A U.S. patent application has been filed on the technology of the new instrument.

OBJECTIVE B: Reduce the microbial contamination in process fluids, e.g., in poultry chillers and chiller brines, by new technologies for the removal of contaminants.

PROGRESS B: A process has been successfully developed that uses filter paper impregnated with diatomaceous earth to filter and pasteurize brine for extended use. This process maintains product safety standards and extends the use of brine in disposal sensitive areas.

IMPACT/TECH TRANSFER B: The process was approved by FSIS for testing in a bacon manufacturing plant. Initial results revealed substantial improvement of brine quality by removing solid particles that included microorganisms. The disinfection efficiency was higher than that achieved by UV light which was inhibited by the presence of organic and inorganic matter.

PUBLICATIONS:

Hernlem, B.J., and L-S. Tsai. 1998. Amperometric titration of chlorine with a modified pH meter/titrator. J. Amer. Water Work Assoc. (submitted).

PATENTS:

Tsai, L-S., B.J. Hernlem and C.C. Huxsoll. Membrane sensor for monitoring chlorine dioxide in food processing water. Provisional Patent application SN 60/069, 152 12/9/97.

QUANTITATIVE DETERMINATION OF PATHOGEN REDUCTION DURING ANIMAL SLAUGHTER AND FOOD PROCESSING TO PROVIDE THE SCIENTIFIC BASIS OF HACCP AND RISK ASSESSMENT

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OBJECTIVE A: To investigate the bacterial levels on swine carcasses during slaughter and dressing.

PROGRESS A: Working with a local swine slaughter plant, swab samples were taken at several sites during the slaughter and dressing process and from three sites on the swine carcass. Initial studies indicated that, after the first washer/polisher, bacterial levels on three sites on the carcass (ham, neck flap, and belly) were similar, and so all further samplings used only swabs from the belly of the carcass. Bacteriological sampling consisted of the total aerobic plate count (APC), total enterics, and heat injured, recovered total enterics. Sampling sites during slaughter and dressing of the carcasses included: after dehairing, before and after the first washer/polisher, after singeing, after the final washer/polisher, after evisceration, and before and after chilling. These studies showed that singeing substantially reduced bacterial numbers; however the final washer/polisher was shown to recontaminate the carcasses. Bacterial levels during the evisceration process remained essentially unchanged. There was a further small decline in viable numbers of bacteria during the carcass chilling operation. With the approval of FSIS, a major experiment was conducted in which the final washer/polisher was taken out of use for a day and results supported the earlier studies.

IMPACT/TECH TRANSFER A: These studies indicated that a visually clean carcass, obtained by use of the final washer/polisher, does not translate to a microbiologically clean carcass. Singeing appears to be a significant critical control point. The processor now has permission to keep the final washer/polisher out of use on a long term basis, and will now allow additional studies to further reduce bacterial levels during the evisceration process.

OBJECTIVE B: To determine the resistance of *Salmonella typhimurium* DT 104 to sanitizers used in food processing plants.

PROGRESS B: The emergence of the multiple antibiotic-resistant *S. typhimurium* DT 104 caused further concern to the food industry when it was suggested that these strains might possess increased resistance to other stresses such as the sanitizers used in food processing plants. Using a microtiter culture plate method, the sanitizer resistance of DT 104 and non-DT 104 strains of *S. typhimurium* was compared. The sanitizers tested included chlorine (HOCl), Perox, Ultra-kleen, and Vigilquat. Our studies showed that both the DT 104 and non-DT 104 strains were equally sensitive to the four sanitizers tested.

IMPACT/TECH TRANSFER B: These studies indicated that, while *S. typhimurium* DT 104 may be a cause for increased concern to physicians in treating patients, these strains can be controlled and eliminated by sanitizers now in use in food processing plants.

OBJECTIVE C: To investigate the use of the *Aeromonas hydrophila* group as a potential process integrity indicator during swine carcass slaughter and dressing.

PROGRESS C: Several sites during carcass dressing, evisceration, and cutting were sampled using sponges and the samples plated onto Starch Ampicillin medium. Very high levels of presumptive colonies were isolated just after the dehairing process, suggesting that this step could represent the source of these bacteria. As expected, singeing decreased their levels, while the final washer/polisher caused small increases in numbers. Isolates were purified using standard bacteriological procedures, verified as belonging to the *A. hydrophila* group and then speciated using biochemical identification schemes. Most cultures isolated from different locations in the processing and handling of the carcasses were identified as *A. hydrophila*. Future work will involve using ribotyping with a Qualicon Riboprinter. This will allow a determination of whether the A. hydrophila isolated from different locations in the processing and handling operations is the same or different subtypes.

IMPACT/TECH TRANSFER C: By identifying the subtypes of *A. hydrophila* isolated, it can then be determined that, if there are different subtypes, then each processing area has its own resident population of *A. hydrophila*. If they are the same subtype, then the processing plant needs to increase its cleaning and sanitation efforts and completely eliminate this bacterium from the different locations in the plant. Since *A. hydrophila* is both a psychrotrophic spoilage bacterium and a putative human pathogen, its presence on fresh pork can represent both a product shelf life concern and a potential foodborne disease hazard.

OBJECTIVE D: To determine surface morphology of bovine connective tissue and fascia, and the site of the attachment of *E. coli* 0157:H7.

PROGRESS D: Scanning electron microscopy (SEM) studies showed that the collagen in the connective tissues were arranged in spaghetti-like bundles. The bacteria (single cells or colonies) selectively attached to the collagen fibrils, not to the muscle fibers. The muscle fibers /bundles and fat globules were wrapped with collagen fibers. The bacteria in turn attached to the collagen fibers surrounding the fat globules although attachment of bacteria was rare in collagen-wrapped fat globules. A high density of bacteria was found in areas with crevices or cavities on the tissue surfaces. There is no evidence of bacterial binding to other tissue components. The density of attached cells was higher with very young cells (2 hr and 4 hr cultures) compared to older cells (8, 16 and 24 hr cultures). This observation suggests greater attachment characteristics of cultures in the lag phase vs the stationary phase. The BIAcore studies also showed higher binding with the 5 hr cells than with the 8 and 24 hour cell surface. Our previous SEM studies using a stationary growth phase and inoculating a more dilute cell suspension with binding time of 20 min failed to show tissue attachment. SEM studies on meat and connective tissues validated the results observed with the BIAcore biosensor studies.

IMPACT/TECH TRANSFER D: These findings indicate that age of the cells at the time of attachment is very critical; in addition, the components of meat tissue surface are also important in permitting bacteria to attach.

OBJECTIVE E: To develop a method to study attachment, inhibition and detachment of *E. coli* 0157:H7 to bovine fascia and connective tissue.

PROGRESS E: Inhibition studies using the BIAcore showed that a group of GRAS status food additives (INH, INI, INK and INL) and non-food additives inhibited binding of a collagen-laminin mixture to the *E. coli* sensor surface. The inhibitors evaluated showed varying ability in blocking the binding of collagen-laminin with *E. coli* surfaces (INL>INK8>INI> INK1>INH). SEM microscopy and cell aggregation studies demonstrated that *E. coli* cells also attached to a purified collagen fiber/network. E.coli cell attachment was different from self-aggregation. Results of our studies on tissue attachment and detachment showed inhibition of *E. coli* O157:H7 attachment to intact meat tissues by use of INL inhibitor. Acid washes further removed the attached microorganism, and an acid wash at a pH of 2.5 also had bactericidal effect. Again, as in the studies with the BIAcore and with SEM, there was increased bacterial attachment with the 5-6 hr culture. Inhibition was studied by coating the decontaminated tissues with 0.2% INL inhibitor before the inoculation and detachment processes. Our preliminary results with *E. coli* showed variable results and further optimization is necessary. Decontamination with 5% hydrogen peroxide was more effective than 200 ppm (0.2%) chlorine.

IMPACT/TECH TRANSFER E: Data from these studies provide preliminary evidence for the inhibitory and detaching effects of INL in bovine fascia and connective tissues. A patent disclosure has been approved by ERRC-NAA.

OBJECTIVE F: To develop a method to study attachment, inhibition and detachment of *Salmonella typhimurium* on chicken skin.

PROGRESS F: Our results indicated that the tissues treated with INL and INK detached more bacteria compared to tissues treated with PBS. INL detached 91% more bacteria compared to tissues treated with PBS alone. INK (0.3%) was a more effective inhibitor than INL (0.2%). The former inhibited 75% of *Salmonella* attachment compared to INL and PBS treated tissues. These inhibitors were not bacteriostatic, but in the second step of the detachment process a lower pH of 2.5 showed a bacteriostatic effect on the detached bacteria, while pH 4.8 allowed the bacteria to survive. No growth was detected in extended incubations of up to 56 hrs at 37°C when plated in BHI agar. The acid used in the current detachment process is not a food grade chemical, and substitutes are being evaluated. Utilizing results from these studies, a modified processing technique to inhibit or detach food pathogens from beef carcass and poultry skin will be proposed.

IMPACT/TECH TRANSFER F: Data from these studies provide supporting evidence for the effectiveness of INL and INK as inhibitors and detaching agents for *Salmonella typhimurium* in poultry skin. A patent disclosure has been approved by ERRC-NAA.

PUBLICATIONS:

Bolton, D., A. Oser, G. Cocoma, S. Palumbo and A. Miller. 1998. Integration of critical control point monitoring and total quality management to reduce fecal contamination on dressed pork carcasses. Food Technol. (submitted)

Laubach, S., J. Rathgeber, A. Oser and S. Palumbo. 1998. Microbiology of the swine head meat deboning process. J. Food Protect. 61:249-252.

Medina, M.B. 1997. SPR Biosensor: Food Science Applications. Food Test. Anal. 3:14-15, 36.

Medina, M.B., and P.F. Fratamico. 1998. Binding interactions of collagen I, laminin and fibronectin with immobilized *E. coli* 0157:H7 using surface plasmon resonance biosensor. Biotechnol. Techn. 12:235-240.

Medina, M.B. 1998. Biosensor studies of the binding of extracellular matrix components with immobilized *Escherichia coli* O157:H7 and inhibition with naturally occurring food additives. Biotechnol. Lett. (submitted)

Palumbo, S., P. Klein, J. Capra, M. Eblen and A. Miller. 1998. Comparison of excision and swabbing sampling methodologies to determine the microbiological quality of swine carcass surfaces. Food Microbiol. (submitted)

Rajkowski, K.T., S. Eblen and C. Laubach. 1998. Efficacy of washing and sanitizing trailers used for swine transport in reduction of *Salmonella* and *Escherichia coli*. J. Food Protect. 61:31-35.

Rajkowski, K.T., and E.W. Rice. 1998. Recovery and survival of *Escherichia coli* O157:H7 in reconditioned wastewater. J. Food Protect. (submitted)

ABSTRACTS:

Medina, M.B. 1998. Biosensor studies of collagen and laminin binding with immobilized *Escherichia coli* O157:H7 and inhibition with naturally occurring food additives. SPIE Conf. Pathog. Detect. Remed. Safe Eating, Boston, MA.

Medina, M.B. 1998. Adhesion mechanisms of enterotoxigenic *Escherichia coli* bacteria and attachment of *E. coli* O157:H7 on extracellular matrix components and tissues. Sci. Technol. 2000 Cong., Manila, Philippines.

Palumbo, S., and A. Pickard. 1998. Effect of chlorine on *Salmonella typhimurium* DT 104. Annu. Amer. Soc. Microbiol.

Palumbo, S., D. Bolton and A.J. Miller. 1998. Microbiological monitoring of swine slaughter and dressing operations. Annu. IAMFES Meet.

Yu, L., and S.A. Palumbo. 1998. Use of *Aeromonas* as an indicator of contamination in a swine slaughtering plant. SPIE Conf. Pathog. Detect. Remed. Safe Eating, Boston, MA.

PATENTS:

Medina, M.B. 1998. Use of naturally occurring food additives in combination with other food chemicals to inhibit attachment of bacterial pathogens to food animal carcasses and also to detach such pathogens from food surfaces. Patent Disclosure (Docket No. 0164.98)

Part III. POST SLAUGHTER PATHOGEN MODELING AND CONTROL

DEVELOPMENT OF MICROBIAL MODELING COMPONENTS FOR USE IN RISK ASSESSMENTS

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CRIS NUMBER: 1935-42000-032
FSIS CODE: E3MM02
CRIS TERMINATION DATE: August, 1998

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OBJECTIVE A: To expand the model for the aerobic growth of *Shigella flexneri* and validate it by comparing the growth kinetics of the bacterium predicted by the model with those observed in foods.

PROGRESS A: The expanded model provides growth kinetics for this bacterium in the lower temperature range, providing data for its behavior in the area where food products might be temperature abused. The growth kinetics in food could be predicted from those generated in the culture system.

IMPACT/TECH TRANSFER A: The expanded model was included in the latest version of the USDA's Pathogen Model Program, Version 5.1 released spring 1998.

OBJECTIVE B: To develop a model for acid inactivation for *S. flexneri* in culture broth.

PROGRESS B: Inactivation of *S. flexneri* is being studied as a function of temperature (4-42°C), pH (2 to 5), and NaCl level (0.5 to 9.0%). Current results indicate that survival increased with decreasing temperature and increasing pH.

IMPACT/TECH TRANSFER B: In addition to being incorporated into an inactivation model for *S. flexneri*, these data provide further support for the acid resistance of this bacterium and suggest that many of the acid conditions found in foods would not inactivate this bacterium.

OBJECTIVE C: To model the effect of induced pH-dependent stationary phase acid resistance on the survival of *Escherichia coli* O157:H7.

PROGRESS C: The protocol involves the use of four strains of *E. coli* O157:H7, five temperatures (37, 28, 19, 12, and 4°C), and five pHs (3, 4, 5, 6, and 7). Initially, the pH will be adjusted with HCl, and in further experiments, the pH will be adjusted with different percentages of lactic, malic, acetic, and citric acid.

IMPACT/TECH TRANSFER C: When completed, this study will provide information on the kinetics of acid inactivation of this acid resistant bacterium, particularly in food systems.

PUBLICATIONS:

Buchanan, R.L., and R.C. Whiting. 1998. Risk assessment: a means for linking HACCP and public health. J. Food Protect.

Buchanan, R.L., M. Golden and R.C. Whiting. 1997. Modeling non-thermal inactivation. Food Technol.

Zaika, L.L., O.J. Scullen and J.S. Fanelli. 1997. Growth inhibition of *Listeria monocytogenes* by sodium polyphosphate as affected by polyvalent metal ions. J. Food Sci. 62:867-869, 872.

Zaika, L.L., and J.S. Fanelli. 1998. Effect of temperature, NaCl and EDTA on growth kinetics, cell morphology and cellular proteins of *Listeria monocytogenes*. (submitted)

Zaika, L.L., J.G. Phillips, J.S. Fanelli and O.J. Scullen. 1998. Revised model for aerobic growth of *Shigella flexneri* to extend the validity of predictions at temperatures between 10 and 19°C. Int. J. Food Microbiol. 41:9-19.

RISK MODELING TO IMPROVE THE MICROBIOLOGICAL SAFETY OF POULTRY PRODUCTS

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CRIS NUMBER: 1935-42000-029
FSIS CODE: E5MA04
CRIS TERMINATION DATE: February, 2000

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OBJECTIVE A: To develop predictive models for growth, survival, and inactivation of *Salmonella* ssp. in poultry products.

PROGRESS A: Twelve serotypes of *Salmonella* from broiler ceca were surveyed for their growth kinetics on cooked chicken breast incubated at 25°C. Collection of kinetic data was completed and the results are being analyzed to determine the variability of lag time and growth rate between strains. Effects of previous sodium chloride concentration (0.5 to 4.5%) on the subsequent growth of *Salmonella typhimurium* on cooked chicken breast incubated at 10 to 40°C was investigated and response surface models for lag time and growth rate as a function of previous sodium chloride and temperature are being developed and validated. Growth kinetics of parent and green fluorescent protein (GFP)-expressing strains of *Salmonella* are being investigated on cooked chicken breast incubated at 25°C to establish GFP-*Salmonella* as a tool for modeling behavior of *Salmonella* in raw and cooked poultry products.

IMPACT/TECH TRANSFER A: Predictive models developed in this research project are incorporated into the *Salmonella* - Risk Assessment Modeling Program for Poultry (*S*-RAMPP) and disseminated to stakeholders by mail and on-site demonstrations. Information about strain variation will assist users of *S*-RAMPP to determine how well the models, developed with *S. typhimurium*, predict growth of other strains of *Salmonella*.

OBJECTIVE B: To develop simulation models for assessing the public health impact of poultry-borne pathogens.

PROGRESS B: Current simulation models in version 1.0 of *S*-RAMPP were made more user-friendly and will be available in version 2.0. A new simulation model, the Food Animal Risk Model for Poultry Pathogens (FARM-PP), is under development. FARM-PP predicts the outcomes from consumption of poultry products contaminated with *Salmonella* and/or *Campylobacter*. Banquet, a consumer education version of FARM-PP, is also under development.

IMPACT/TECH TRANSFER B: Assembling current food safety knowledge in the form of user-friendly simulation models has great potential for assisting the poultry industry and regulatory agencies in making critical food safety decisions that impact public health and in educating the public about food safety. Version 2.0 of *S*-RAMPP and version 1.0 of FARM-PP will be released in December of 1998. Seventy-five copies of *S*-RAMPP version 1.0 were distributed worldwide in 1998.

PUBLICATIONS:

Oscar, T.P. 1997. Predictive modeling for risk assessment of microbial hazards. Reciprocal Meat Conf. Proc. 50:98-103.

Oscar, T.P. 1997. Use of computer simulation modeling to predict the microbiological safety of chicken. Proc. 32nd Nat. Meet. Poultry Health Proc. 73-83.

Oscar, T.P. 1998. Identification and characterization of *Salmonella* isolates by automated ribotyping. J. Food Protect. 61:519-524.

Oscar, T.P. 1998. Growth kinetics of *Salmonella* isolates in a laboratory medium as affected by isolate and holding temperature. J. Food Protect. 61:964-968.

STRESS ADAPTATION AND VIRULENCE EXPRESSION OF BACTERIAL PATHOGENS IN FOOD ENVIRONMENTS

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OBJECTIVE A: Develop an effective process to eliminate *Listeria monocytogenes* from ready-to-eat meat and poultry.

PROGRESS A: *L. monocytogenes* has the ability to grow at elevated salt concentrations and at low temperatures. A variety of commonly occurring plant and animal compounds that *L. monocytogenes* can utilize to overcome or adapt to conditions of elevated NaCl concentration or refrigeration temperature were identified. Glycine betaine, proline betaine, acetyl carnitine, carnitine, γ-butryobetaine, and 3-dimethylsulfoniopropionate have been identified as osmoprotectants and cryoprotectants for *L. monocytogenes* as evidenced by an increase in growth rate of the organism when provided with these compounds while being salt or chill-stressed in defined medium. The presence of osmoprotectants and cryoprotectants in foods is likely to help bacteria overcome the barriers of high osmotic strength and low temperature designed to control microbial growth. Cold shock was used as a means to reduce the thermal tolerance of *L. monocytogenes* in a variety of food products. *L. monocytogenes* and *L. innocua* strains exhibited *D* values that were depressed 13-37% after 3 h cold shock at 0°C. Additionally, cold shock can reduce the thermal tolerance of *L. monocytogenes* on frankfurter skins by 25% and in milk by 15%. Fatty acid analysis and differential scanning calorimetry (DSC) were used in an effort to determine the basis for the cold shock-induced reduction in thermal tolerance of *L. monocytogenes*. Fatty acid profiles showed no significant variations as a result of cold shock, indicating that changes in membrane fatty acids were not responsible for the cold shock induced reduction in thermal tolerance. DSC measurements show that cold shock produces alterations in the cellular components responsible for protein synthesis (ribosomes) in the cells. The data suggests that either 50S subunit or 70S particle sensitivity was responsible for the intact ribosome fragility. These changes in the ribosomes are correlated with a reduction in the organism's ability to withstand heat treatment.

IMPACT/TECH TRANSFER A: Identification of commonly occurring plant and animal compounds that *L. monocytogenes* can utilize to overcome or adapt to conditions of high salt concentration or refrigeration temperature will assist in identifying foods where added measures may be needed to control or eliminate the pathogen.

OBJECTIVE B: Develop simplified systems to study virulence of *Yersinia enterocolitica* in the mouse model and maximize stability of the virulence plasmid and of expression of plasmid-encoded proteins allowing the design of improved detection assays.

PROGRESS B: The effects of iron-dextran and the iron chelator, deferoxamine mesylate (Desferal), on the course and outcome of experimental yersiniosis were investigated in mice using pathogenic *Y. enterocolitica* isolates from artificially and naturally contaminated food samples. Experiments in which plasmid-bearing *Y. enterocolitica* food isolates were given orally to mice showed that the virulence and the median dose of oral infection were not affected by conventional pretreatment with iron-dextran and Desferal. Thus, a simplified, effective mouse virulence assay was developed which did not require iron-pretreatment. Due to the unstable nature of the virulence plasmid in pathogenic *Y. enterocolitica*, detailed studies were performed which established conditions for maximization of plasmid stability and expression of plasmid-encoded proteins in *Y. enterocolitica*. These observations suggest new approaches for rapid isolation of plasmid-bearing virulent strains of *Y. enterocolitica* from food samples.

IMPACT/TECH TRANSFER B: These improved technologies will be provided to FSIS Microbiology Division through hands-on demonstration and a detailed written description. The Specialty Laboratories, Los Angeles, is interested in direct application of PCR for detection of pathogenic *Y. enterocolitica*.

OBJECTIVE C: Sequence and determine the arrangement of the genes in the antibiotic resistance gene cluster of *Salmonella typhimurium* DT104.

PROGRESS C: The mechanism by which *S. typhimurium* DT104 accumulated resistance genes is of interest because these genes interfere with treatment of infections caused by this organism. The genes might be horizontally transferred to other bacteria, even to unrelated organisms. The arrangement and the location of the integrons and other resistance genes have been determined. A chloramphenicol resistance gene (*cmlA*) appears to be homologous to a *cmlA* exporter gene found in *Pseudomonas aeruginosa*. Both type I integrons possess the classical structure, as evidenced by the presence of a 5' integrase gene and a 3' *sulI* gene, separated by an antibiotic resistance gene and a disinfectant resistance gene, $qacE\Delta$. In addition, the integron arrangement is shown to be useful for PCR detection of multi-resistant *S. typhimurium* DT104.

IMPACT/TECH TRANSFER C: Knowledge of the arrangement of the antibiotic resistance genes in *Salmonella typhimurium* DT104 and the mechanism of acquisition could have an impact on antibiotic use in agriculture.

OBJECTIVE D: Develop an improved multiplex PCR for specific identification of *Escherichia coli* O157:H7.

PROGRESS D: In order to simplify specific testing for *E. coli* O157:H7 allowing identification of the H type and of the type of toxin produced (Stx1, Stx2 or both), a multiplex PCR was designed. Primers for the *fliC* (encodes H7 flagellar antigen), stx_1, stx_2, and *eaeA* genes and a 60-MDa plasmid sequence were used in a multiplex PCR for simultaneous amplification of the five sequences. The PCR allowed detection of the organism in seeded foods and bovine feces after enrichment culturing. Use of the multiplex PCR shortens the time required to confirm *E. coli* O157:H7 isolates and allows determination of the type of toxin produced.

IMPACT/TECH TRANSFER D: The multiplex PCR allows for rapid, specific detection and identification of *E. coli* O157:H7 in various types of foods and other samples. The technology will be transferred to FSIS Microbiology Division through demonstration and written description of the procedure.

PUBLICATIONS:

Bayles, D.O., and B.J. Wilkinson. 1998. Osmoprotectants and cryoprotectants for *Listeria monocytogenes*. J. Food Protect. (submitted).

Bayles, D.O., M.H. Tunick, T.A. Foglia and A.J. Miller. 1998. Cold shock and its effect on ribosomes and thermal tolerance in *Listeria monocytogenes*. Appl. Environ. Microbiol. (submitted).

Bhaduri, S., and B. Cottrell. 1997. Direct detection and isolation of plasmid-bearing virulent serotypes of *Yersinia enterocolitica* from various foods. Appl. Env. Microbiol. 63:4952-4955.

Bhaduri, S., and B. Cottrell. 1998. A simplified sample preparation method from various foods for PCR detection of pathogenic *Yersinia enetrocolitica*: a possible model for other food pathogens. Mol. Cell. Probes. 12:79-83.

Briggs, C.E., and P.F. Fratamico. 1998. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. Antimicrob. Agents Chemother. (submitted).

Fratamico, P.M., and T.P. Strobaugh. 1998. Evaluation of an enzyme-linked immunosorbent assay, direct immunofluorescent filter technique, and multiplex polymerase chain reaction for detection of *Escherichia coli* O157:H7 seeded in beef carcass wash water. J. Food Protect. 61:934-938.

Fratamico, P.M., and T.P. Strobaugh. 1998. Simultaneous detection of *Salmonella* spp. and *Escherichia coli* O157:H7 by multiplex PCR. J. Indust. Microbiol. Biotechnol. (in press)

Fratamico, P.M., and T.P Strobaugh. 1998. Detection of *Escherichia coli* O157:H7 using a surface plasmon resonance biosensor. Biotechnol. Techn. (in press)

Haiyu L., S. Bhaduri and W.E. Magee. 1998. Maximizing plasmid stability and production of released proteins in *Yersinia enterocolitca.* Appl. Environ. Microbiol. 64:1812-1815.

Janisiewicz, W.J., W.S. Conway, M. Brown, G.M. Sapers, P.M. Fratamico and R.L. Buchanan. 1988. Fate of *Escherichia coli* O157:H7 on fresh cut apple tissue and its transmission by fruit flies. Appl. Envrion. Microbiol. (submitted).

ABSTRACTS:

Bayles, D.O., and A.J. Miller. 1998. Ribosomal enthalpy changes resulting from cold shock of *Listeria monocytogenes.* Annu. Amer. Soc. Microbiol. K106.

Bhaduri, S., and B. Cottrell. 1998. Simplification of a mouse virulence test for evaluation of pathogenicity of plasmid-bearing virulent *Yersinia enterocolitica* from food samples. Annu. Soc. Indust. Microbiol. P26.

Briggs, C.E., and P.M. Fratamico. 1998. The effect of antibiotic stress on RpoS and heat shock protein expression in multiple antibiotic resistant *Salmonella typhimurium* DT104. Gordon Conf. Microbial Stress Responses, Henniker NH, 22.

ASSURANCE OF MICROBIOLOGICAL SAFETY OF THERMALLY PROCESSED FOODS

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OBJECTIVE A: Define the heat treatment required to achieve a specified lethality for *Escherichia coli* O157:H7 in media system to ensure that the heating step is lethal, while avoiding heating that negatively impacts product quality. Use data to develop a mathematical model for determining the effects of environmental parameters on thermal resistance of *E. coli* O157:H7.

PROGRESS A: Initial data were collected on the heat resistance of a mixture of *E. coli* O157:H7 in media system. Environmental parameters assessed were pH (4.0 - 8.0), sodium chloride (0 - 6%), and sodium pyrophosphate (0 - 0.3%). Surviving cells were determined using plate count agar supplemented with 1% sodium pyruvate. Decimal reduction times (D-values) were calculated by fitting a survival model to the data with a curve fitting program. The D-values were analyzed by second order response surface regression equation in temperature, pH, sodium chloride and sodium pyrophosphate levels. The four variables interacted to affect the inactivation of the pathogen. Confidence intervals (95%) were developed to allow microbiologists to predict lethality of heat to *E. coli* O157:H7. Thermal resistance of *E. coli* O157:H7 can be lowered by combining these intrinsic factors.

IMPACT/TECH TRANSFER A: The multiple regression equation, developed in this study, can predict D-values for any combinations of temperature, salt, sodium pyrophosphate, and pH that are within the range of those tested. Using this predictive model, food processors should be able to design thermal processes for the production of a safe food with extended shelf life without substantially adversely affecting the quality of the product.

OBJECTIVE B: Develop a model to predict the growth of *Clostridium perfringens* from spores at temperatures applicable to the cooling of cooked meat.

PROGRESS B: The effect of temperature (15-51°C) on the growth from a spore inoculum of a three strain mixture of *C. perfringens* were determined. The growth medium used was trypticase-peptone-glucose-yeast extract broth. *C. perfringens* populations were determined at appropriate

intervals by plating onto tryptose-sulfite-cycloserine agar. *C. perfringens* growth from spores was not observed at a temperature of 51° C for up to 3 weeks. It was found that, generally, the logistic function provided a better prediction of relative growth of more than ½ $\log_{10}$ than that of the Gompertz function. Using the logistic function the two parameters: germination, outgrowth, and lag (GOL) time; and exponential growth rate, EGR, were determined. The exponential growth rates and the reciprocal of the GOL times were fitted to the square root Ratkowsky model, using temperature as the independent variable. Applying multivariate statistical procedures, confidence intervals were computed on the prediction of the amount of growth for a given temperature. Closed form equations are developed that allow for predicting growth for a general cooling scenario and the standard error of the prediction. These equations depend upon microbiological assumptions of the effect of history of the GOL times for gradual changes in temperature.

IMPACT/TECH TRANSFER B: The findings, addressing FSIS needs, were sent to FSIS-Standards Development Branch which used the information to aid with the disposition of products subject to cooling deviations.

OBJECTIVE C: Develop a predictive model for proteolytic *Clostridium botulinum* growth at 12 to 48°C simulating the cooling of cooked meat.

PROGRESS C: Germination, outgrowth and lag (GOL), and exponential growth rates of *C. botulinum* from spores at temperatures (12-48°C) applicable to the cooling of cooked meat products were determined. The growth medium, Reinforced clostridial medium (RCM) supplemented with oxyrase enzyme to create suitable anaerobic conditions, was inoculated with approximately 4 $\log_{10}$ spores/ml. *C. botulinum* populations were determined at appropriate intervals by plating onto RCM. *C. botulinum* growth from spores was not observed at temperatures < 12 °C or > 48°C for up to 3 weeks. Growth curves were determined by fitting Gompertz functions to the data. From the parameters of the Gompertz function the growth characteristics, GOL times and exponential growth rates were calculated. These growth characteristics were subsequently described by Ratkowsky functions using temperature as the independent variable. Closed form equations were developed that allow for predicting relative growth for a general cooling scenario. By applying multivariate statistical procedures, the standard errors and confidence intervals were computed on the predictions of the amount of relative growth for a cooling scenario. The predictive model is capable of predicting spore outgrowth and multiplication for general cooling scenarios.

IMPACT/TECH TRANSFER C: The findings, addressing FSIS needs, were sent to FSIS-Standards Development Branch which used the information to aid in evaluating the safety of cooked product after cooling.

OBJECTIVE D: Define the heat treatment required to achieve a specified lethality for *Salmonella* spp. in chicken broth, beef, pork, turkey and chicken.

PROGRESS D: The heat resistance of 35 *Salmonella* spp. isolated from different meat species was determined at 55 to 65°C in chicken broth, beef, pork, turkey and chicken. Thermal death times were determined using a submerged-coil heating apparatus. The surviving cell population was determined by spiral plating heated samples onto tryptic soy agar supplemented with 0.6% yeast extract and 1% sodium pyruvate. D-values in chicken broth at 55°C ranged from 5.86 min for *S. copenhagen* 8457 (pork isolate) to 3.77 min for *S. hadar* MF60404 (Turkey isolate); the D-values at 62°C were 0.40 and 0.32 min, respectively. The D-values of a mixture of 8 strains, representing isolates from each species of meat and poultry exhibiting highest heat resistance, were 4.87, 2.72, 1.61 and 0.41 min at 55, 58, 60 and 62°C. No correlation between the heat resistance and the origin of the *Salmonella* spp. could be established due to significant variation in the heat resistance among strains. The z-values of all strains including the cocktail were very similar, ranging from 6.85 to 5.77 C°. The D-values of the 8 *Salmonella* spp. Cocktail in beef were 7.93, 2.34, 1.50, and .67 min; in pork were 10.42, 2.27, 1.62, and 0.87 min; in turkey were 9.27, 2.27, 1.51, and .80 min; in chicken were 8.96, 2.11, 1.36, and 0.59 min at 58, 60, 62.5, and 65°C, respectively.

IMPACT/TECH TRANSFER D: Understanding these variations in heat resistance should assist food processors to ensure and to design adequate thermal regimes to eliminate *Salmonella* in thermally processed beef, pork, turkey and chicken. Thermal death time values from this study were sent to FSIS for the development of lethality performance standards.

OBJECTIVE E: Quantify the heat resistance of *Salmonella* spp. in chicken breast and thigh meat.

PROGRESS E: The pH of the meat is one of the most important factors influencing the microbial heat resistance. Since the chicken breast muscles have a lower pH than the thigh, it was logical to determine the heat resistance of *Salmonella* spp. in chicken breast and thigh meat. When the surviving bacteria were enumerated on tryptic soy agar supplemented with 0.6% yeast extract and 1% pyruvate (non-selective medium), D-values in chicken breast meat were 6.08, 4.77, 3.00 and 0.66 min at 55, 57.5, 60, and 62.5°C, respectively; the values in thigh meat ranged from 11.48 min at 55°C to 0.84 min at 62.5°C. As expected, the measured heat resistance was lower when the recovery medium was selective (xylose lysine deoxycholate agar).

IMPACT/TECH TRANSFER E: Thermal death time values from this study will assist food processors in designing acceptance limits on critical control points that ensure safety against *Salmonella* spp. in chicken meat.

PUBLICATIONS:

Juneja, V.K., O.P. Snyder, A.C. Williams and B.S. Marmer. 1997. Thermal destruction of *Escherichia coli* O157:H7 in hamburger. J. Food Protect. 60:1163-1166.

Juneja, V.K., P.G. Klein and B.S. Marmer. 1997. Heat shock and thermotolerance of *Escherichia coli* O157:H7 in a model beef gravy system and ground beef. J. Appl. Microbiol. 84:677-684.

Juneja, V.K., T.A. Foglia, and B.S. Marmer. 1997. Heat resistance and fatty acid composition of *Listeria monocytogenes:* Effect of pH, acidulant, and growth temperature. J. Food Protect. 61:683-687.

Juneja, V.K., and H.M. Marks. 1998. Proteolytic *Clostridium botulinum* growth at 12 to 48°C simulating the cooling of cooked meat: development of a predictive model. Food Microbiol. (submitted).

Juneja, V.K., and B.S. Marmer. 1998. Lethality of heat to *Escherichia coli* O157:H7: D- and z-values lamb and pork determinations in turkey. J. Food Safety (submitted).

Juneja, V.K., and B.S. Marmer. 1998. Thermal inactivation of *Clostridium perfringens* vegetative cells in ground beef and turkey as affected by sodium pyrophosphate. Food Microbiol. (in press)

Juneja, V.K., R.C. Whiting, H.M. Marks and O.P. Snyder. 1998. Predictive model for growth of *Clostridium perfringens* at temperatures applicable to cooling of cooked meat. Food Microbiol. (submitted).

OPTIMIZATION OF THE SAFETY, QUALITY, AND SHELF-LIFE OF IRRADIATED POULTRY AND RED MEAT

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OBJECTIVE A: Determine gamma radiation sensitivitiy of *Clostridium perfringens* vegetative cells and endospores on ground beef.

PROGRESS A: This study was initiated because we observed survival of *C. perfringens* during a study of PCR and DNA hybridization detection following doses of gamma radiation that would be expected to eliminate vegetative cells and in some cases spores of other clostridia. We have confirmed that the radiation resistance of both the vegetative cells and the spores are much greater than those expected of *C. botulinum*. Our results differ from published values, and as a result we are conducting studies with the vegetative cells and spores of several indivdual isolates and serotypes to ensure validity.

IMPACT/TECH TRANSFER A: Unknown, but if our preliminary results are correct, they may have implications for both the sterilization of food and medical goods.

OBJECTIVE B: Determine if there is an oxygen effect in the presence of 50% CO_2 on the inactivation of *Listeria monocytogenes* by gamma irradiation and on the subsequent multiplication of survivors at 10°C on turkey meat.

PROGRESS B: The radiation resistance was inversely related to the oxygen content of the atmosphere during irradiation. The gamma-radiation D-values for the inactivation of a mixture of four isolates of *L. monocytogenes* on ground turkey were 0.80, 0.74, 0.75, 0.76, 0.70, and 0.64 kGy in modified atmospheres including 50 % CO_2, nitrogen, and 0, 5, 10, 15, 20, and 25% O_2, respectively. Ground turkey meat was inoculated with approximately 4 x 10^3 colony forming units (CFU)/ gram and packaged in the modified atmospheres described above, irradiated with a dose of 1.5 kGy, and stored at a modest abuse temperatue of 10°C. The decrease in CFU/gram from the intital treatment with irradiation was greatest at the higher oxygen concentrations, though there were survivors in every case. During storage for 27 days the CFU in the samples irradiated in atmospheres of 50% CO_2 and 50% N_2; 50% CO_2, 45% N_2, and 5% O_2; 50% CO_2, 40% N_2, 10% O_2;

50% CO_2, 35% N_2, and 15% O_2; and 50% CO_2, 30% N_2, and 20% O_2; multipled and reached final populations of 7.59, 6.34, 5.70, 2.13, and 3.13 log CFU/g, respectively. MAP atmospheres containing either 15 or 20% O_2 and 50% CO_2 with a very low radiation dose of 1.5 kGy significantly increased the shelf-life of the ground turkey.

IMPACT/TECH TRANSFER B: The results of this study indicate that much better control of *L. monocytogenes* can be obtained by irradiation in the presence of oxygen. It is unclear however if a modified atmosphere containing CO_2 and O_2 will influence the acceptability of the meat. Additional studies will be required to determine the influence of such atmospheres on the sensorial properties of the irradiated meat.

OBJECTIVE C: Determine if the shelf-life of lamb meat can be extended by a combination of low-dose gamma irradiation and refrigerated storage under a modified atmosphere containing CO_2, N_2, and O_2.

PROGRESS C: Ground lamb was packaged in air-permeable packaging or in modified atmospheres within a barrier package of 50% CO_2, 5% O_2, and 45% N_2; 50% CO_2, 15% O_2, and 35% N_2; 50% CO_2, 25% O_2, and 30% N_2; and 50% CO_2 and 50% N_2. One-half of the samples were irradiated to a dose of 1.5 kGy; the others were not irradiated. All samples were stored at a temperature of 2°C and sampled weekly to determine the total aerobic plate counts of the meat over a period of 15 weeks. The initial plate counts were approximately 10^6 and 10^3 CFU/g in the non-irradiated and irradiated samples, respectively. In air-permeable packaging the aerobic plate count increased rapidly even in the irradiated samples. The irradiated samples were less than 10^7 CFU/gram for approximately 28 days, whereas some of the non-irradiated samples exceeded 10^7 CFU/gram within one week. An atmosphere of 50% CO_2 and 50% N_2 restricted to below 10^7 CFU/gram the multiplication of the indigenous microflora of the lamb for a period of approximately 12 weeks at 2°C. Irradiated samples in an atmosphere of 5% O_2 reached approximately 10^6 CFU/gram in four weeks. The log CFU/g values of irradiated samples after storage for 14 weeks at 2°C were 5.45, 1.84, and 4.49 for samples in MAP containing 5, 15, and 25% O_2, respectively. On the basis of the aerobic plate counts all of these samples were microbiologically acceptable.

IMPACT/TECH TRANSFER C: These results indicate that the use of a modified atmosphere, a low radiation dose of 1.5 kGy, combined with a good refrigeration storage temperature can significantly increase the shelf life of very labile products such as ground meat.

OBJECTIVE D: Determine the effect of temperature (-76 to +10C) on the gamma radiation resistances of *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* on ground beef and to develop predictive models for the increase in radiation resistance associated with decreased processing temperature.

PROGRESS D: This study was initiated because of the report of industrial experiences in the inability to maintain uniform temperatures in frozen products during irradiation sterilization. The intent is to provide sufficient data to allow the D-values for inactivation of at least two Gram-negative and two Gram-positive foodborne pathogens to be determined from the the temperature of dry ice to 10°C. These studies will eventually be used to expand the predictive equations already available in the USDA Predictive Modeling program. Studies of the effects of temperature during irradiation have been completed for *E. coli* O157:H7 and a mixture of 5 *Salmonella* spp.

IMPACT/TECH TRANSFER D: The results are expected to provide much better guidance to processors and regulators for the prediction of the inactivation of foodborne pathogens in deep frozen meats.

OBJECTIVE E: Determine the effect of ionizing radiation dose rate (gamma versus electron beam) on the inactivation of *Salmonella tyhpimurium* Type DT104 on meat.

PROGRESS E: This study represents a recently initiated cooperative effort with Iowa State University to determine the D-values of *S. typhimurium* DT104 using the same meats and cultures in both laboratories. Iowa State will use electron-beam technology, and ARS will use gamma radiation.

IMPACT/TECH TRANSFER E: Two objectives will be met by this study, determining the effects of dose rate and fat content on D-values of an important foodborne pathogen.

OBJECTIVE F: Determine the growth conditions that lead to the maximum pH-dependent stationary phase, acid tolerance response in *E. coli* O157:H7.

PROGRESS F: The acid-tolerant response of 4 strains of *E. coli* O157:H7 grown under aerobic or anaerobic conditions in the presence or absence of 11 different fermentable carbohydrates was measured by assaying for survival in Brain Heart Infusion broth at pH 2.5. Most sugars induced a survial rate of at least 80% after 3 hours of exposure to acidic conditions. However, after 6 hours glucose is the only sugar that allowed for survival of > 75% of the bacteria in acidic conditions. Of the 11 carbohydrates tested, sucrose inhibited the acid tolerant response with 0 to < 25% of the cells surviving acidic conditions after 3 hours. Under anaerobic conditions the acid tolerant response is increased by an approximately 10% greater survival rate when bacteria are grown with glucose. Growth in glucose to induce the acid tolerant response also leads to biofilm production in the strains tested. If cells are grown in a medium adjusted to pH 5.0 with HCl there is no induction of the acid-tolerant response. If cells are grown in buffered media at pH 8.0 with glucose there is a moderate acid-tolerant response; therefore, a product of glucose fermentation must be the inducer of this response. Formic acid does not induce the acid tolerant response.

IMPACT/TECH TRANSFER F: Provides information about the effect of growth conditions on the acid tolerant response and possible cross protection to food processing technologies.

OBJECTIVE G: Isolate rpoS mutants to differentiate pH dependent and pH independent stationary phase acid tolerant response in *E. coli* O157:H7.

PROGRESS G: Clinical isolates of *E. coli* O157:H7 were screened for catalase activity. Of nine strains tested, two had decreased activity. All nine isolates were assayed for production of rpoS using a western blot. One of the two catalase negative isolates did not express rpoS. Using Southern blot analysis this strain did not contain the rpoS gene. The gene from the strain that expressed rpoS was obtained by PCR, and the DNA sequence was determined. This strain contains a mutation that changes amine acid 33 from a leucine to a glutamic acid. An acid phosphatse assay was used to test for functional rpoS in this strain and was negative, indicating a nonfunctional rpoS. These two strains also do not produce curli protein, which is known to be transcribed by rpoS. These strains were then used to assay the pH dependent stationary acid tolerant response. It was found that glucose induces this response in the absence of rpoS. Thirty-five more strains have been screened for HPII activity and there are 7 more possible rpoS mutants that will be characterized further.

IMPACT/TECH TRANSFER G: These rpoS mutants offer a means to study the pH dependent acid tolerant response and to determine if this response can lead to cross protection of acid tolerant cells to ionizing radiation or heat.

OBJECTIVE H: Develop an ASTM guide for dosimetry in radiation research on food and agricultural products.

PROGRESS H: A standard guide was developed in ASTM Committee E10.01 that covers the minimum requirements for dosimetry and absorbed-dose validation needed to conduct research on the irradiation of food and agricultural products. Such research includes establishment of the quantitative relationship between the absorbed dose and the relevant effects in these products. This guide also describes the overall need for dosimetry in such research, and in reporting of the results. It is intended for use by research scientists in the food and agricultural communities, and not just scientists conducting irradiation research. It, therefore, contains more tutorial information than most other ASTM dosimetry standards for radiation processing. The effects produced by ionizing radiation in biological systems depend on a large number of factors which may be physical, physiological, or chemical. Although not treated in detail in this guide, quantitative data of environmental factors that may affect the absorbed-dose response, such as temperature and moisture content in the food or agricultural products, should be reported. The uncertainty in the absorbed-dose measurement is described. The guide covers research conducted using the following types of ionizing radiation: gamma rays, bremstrahlung X-rays, and electron beams.

IMPACT/TECH TRANSFER H: When the guide becomes publically available in 1998 it could help scientists and industry to obtain much greater repeatability in both research and industrial treatments.

OBJECTIVE I: To test the concept that ionizing radiation treatments will produce characteristic changes in the thermodynamic properties of actomyosin of meat that can be identified by differential scanning calorimetry.

PROGRESS I: This PL480 project with a cooperating scientist in Poznan, Poland, was initiated this year. Over 150 analyses of irradiated broiler breast meat have been completed. Preliminary results indicate that there is an effect of irradiation on the thermodynamic properties of the chicken actomyosin but that the effect may not be stable during post irradiation storage.

IMPACT/TECH TRANSFER I: Unknown until the study has been completed.

OBJECTIVE J: Identify the effects of ionizing radiation on food phytate and phytate containing materials that are commonly used to extend hamburger in the USDA School Lunch Program (SCA, Howard University).

PROGRESS J: Current consumer preferences for plant and plant/meat products are a nutritional concern because phytate (inositol hexaphosphate, phytic acid or IP6) chelates minerals, preventing their abosorption into the body. As phosphate groups are hydrolyzed from inositol by traditional food processes, a linear reduction in mineral binding capacity results. In an attempt to further hydrolyze phytate, this study found that the irradiation of sodium phytate, soy, soy-extended beef, and beef at an absorbed dosage of 3.4 to 3.8 kilogray (kGy) did not cause a reduction ($p>0.05$) in the level of IP3 hydrolysis products. Anion-exchange spectroscopy and high performance liquid chromatography were the analytical methods used; however, results differed slightly. There is a need for improved inositol phosphate detection methods applicable both to human and animal foods.

IMPACT/TECH TRANSFER J: These results help to demonstrate the lack of an adverse nutritional effect of irradiating soy-extended beef like that used in the USDA School Lunch Program.

OBJECTIVE K: Determine the effects of irradiating soy-extended ground beef patties on their microbial load and nutritional value (SCA, Howard University).

PROGRESS K: Hamburger for the school lunch program is often extended with soy, which may bind essential minerals. The ground beef may be contaminated with foodborne pathogens. The effect of gamma irradiation (4 kGy dose) on the microbial population of soy-extended ground beef and its nutritional value was investigated using hamburgers prepared for the school-lunch program

in a 3 week feeding study with 84 weanling rats. There were no differences in liver or bone mineral concentrations, nor effects on body weight, feed efficiency, survival of experimental animals, nor in any of four blood or seven biochemical parameters measured. Microbiological tests indicated that a radiation dose of 4 kGy almost completely eliminated the bacterial population of the meat yet had no negative nutritional effects. The study indicates the potential value of ionizing irradiation to control foodborne pathogens on the hamburger used in the school lunch program.

IMPACT/TECH TRANSFER K: This data expands the existing data on the effectiveness of ionizing irradiation to control foodborne pathogens on ground beef to include soy-extended products and further indicates that there are no adverse nutritional changes as a result of the treatment.

OBJECTIVE L: Test the concept of chemiclearance by evaluation of the effect of ionizing radiation on vitamin content of exotic meats as compared to the effects of similar treatments in domesticated animal tissue.

PROGRESS L: Changes in thiamin, riboflavin and α-tocopherol concentrations due to gamma irradiation were followed in alligator, caiman, bison and ostrich (exotic) meats. The proximate composition showed that the exotic meats generally had lower fat content than domestic animals and the thiamin content of the reptiles was lower. The changes in the vitamins due to irradiation were similar to those previously observed for domestic species. The results indicate that the loss of vitamins in these species is negligible insofar as the American diet is concerned, and that the concept of "chemiclearance" is applicable to exotic meats.

IMPACT/TECH TRANSFER L: The results of this study were included in the data considered by the FDA in approval of the irradiation of red meats.

PUBLICATIONS:

Anonymous. 1998. (D.W. Thayer served as Co-Chair for this Committee). E1900-97: Guide for dosimetry in radiation research on food and agricultural products. 1998 Annu. Book ASTM Standards. 12.02:1-9.

Dubey, J.P., D.W. Thayer, C.A. Speer and S.K. Shen. 1998. Effect of gamma irradiation on unsporulated and sporulated *Toxoplasma gondii* oocysts. Int. J. Parasitol. 28:369-375.

Engeljohn, D.L., D.W. Thayer and B.F. Harland. 1999. Effects of ionizing radiation on food phytate. J. Food Composition Anal. (in press).

Engeljohn, D.L., B.B. Bell, D.W. Thayer and B.F. Harland. 1999. Effects of gamma irradiated, cooked, soy-, beef-, and soy-meat-based diets in rats. Ecol. Food Nutr.. (in press).

Fox, J.B., and D.W. Thayer. 1998. Radical oxidation of riboflavin. Int. J. Vit. Nutrit. Res. 68:174-180.

Lakritz, L., J.B Fox and D.W. Thayer. 1999. Thiamin, riboflavin and α-tocopherol content of exotic meats and loss due to gamma radiation. J. Food Protect. 62: (in press).

Schwartz, D., L. Lakritz and K. Kohout. 1996. pp. 410-411. *In:* C.H. McMurray, E.M. Stewart, R. Gray, and J. Pierce (eds.), Detection methods for irradiated foods. Roy. Soc. Chem., Cambridge, UK.

Thayer, D.W. 1998. Regulatory status of irradiation of meat and poultry. Reciprocal Meat Conf. Proc. 51:164-165.

Thayer, D.W., G. Boyd, A. Kim, J.B Fox Jr., and H.M. Farrell Jr. 1998. Fate of gamma irradiated *Listeria monocytogenes* during refrigerated storage on raw or cooked turkey breast meat. J. Food Protect. 61:979-987.

ABSTRACTS:

Thayer, D.W. 1997. Development of irradiated shelf-stable meat and poultry products. NFPA/NASA Workshop on development of a commercial space center for food technology. Glennview, IL.

Thayer, D.W. 1997. Scientific basis of the isomedix petition to the FDA to approve the irradiation of meat from domesticated mammalian species. Proc. AMI-Meat Indust. Res. Conf.

Thayer, D.W., J.G. Phillips and G. Boyd. 1998. Computer models for radiation inactivation of *S. typhimurium* and *E.coli* O157:H7 on meat and poultry. Proc. 2nd FAO/IAEA Res. Co-ordin. Meet. Develop. Shelf-stable and Ready-to-eat Food High-dose Irradiat Process. Beijing, China.

DEVELOPMENT OF MINIMALLY DEGRADATIVE PASTEURIZATION PROCESSES FOR LIQUID OR SOLID FOODS

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OBJECTIVE A: Using surface pasteurization, economically reduce microbial contamination on the surface of solid foods (e.g., poultry) without significant loss of product quality.

PROGRESS A: Rather than inoculating bacteria on the carcass surface we emphasized reduction of naturally occurring bacteria on the products. We determined approximate optimum process parameters using chicken halves. The optimum parameters are initial vacuum 0.1 sec., final vacuum 0.5 sec., steam time 0.1 sec., and steam temperature 126-138C. We applied the process to farm raised catfish, turkey drumsticks, pork chops, and hot dogs. Treatment of pork chops at 126C resulted in greater than 1 log kill of bacteria by APC. Because hot dogs are a cooked product, we inoculated with non-pathogenic *Listeria*. The process at 138C essentially destroyed all bacteria on hot dogs. The catfish and turkey results are not available.

We attempted to verify the ability of the surface pasteurizer to reduce the bacteria levels on the surface of chicken received directly from Federally inspected processing plants. The tests and follow-up experiments showed the cavity was not treated. Bacteria were killed on the exterior surface and we are modifying the unit to assure treating the cavity. With the exception of the cavity, the surface pasteurizer reduced *E. coli* levels to less than 2 log. Kills for *E. coli*, coliforms, and APC were generally 0.6 to 1.0 log. Although we expect the unit to be placed in the process line before the chiller, these tests were for birds which had been chilled and shipped over night with ice packs.

IMPACT/TECH TRANSFER A: During the year, a news release, eight interviews, a trade level presentation, three manuscripts and three professional presentations were partially responsible for the formation of a Trust Fund Cooperative Agreement, two Confidentiality and MTA agreements, and two pending CRADAs. Four industrial entities are interested in applying the surface pasteurization of fresh broilers to commercial operation, to reduce or eliminate surface contamination by *E. coli* and *Salmonella*, using only steam and without quality degradation.

Cooperative testing is underway, and designs are being prepared to build a mobile pasteurizer to run tests at the processors' sites. If successful, this offers the possibility of much safer chicken products, at very little additional cost, and no environmental or safety issues resulting from the use of biocides. In addition, cooperation is underway with Mississippi State University to reduce spoilage organisms on catfish using the same technology. Three patent disclosures were approved by the ARS patent committee, but have not been filed yet.

OBJECTIVE B: Develop new electrical pasteurization technology applicable at lower temperatures for temperature sensitive liquid foods such as liquid egg.

PROGRESS B: Developed a turbulent flow process using the double pipe/microwave system with high recirculation within the microwave to assure turbulent flow and no recycle. The process isolates thermal and non-thermal effects to test destruction of microorganisms at low temperatures. Combinations of yeast, *Pediococcus sp*, *E. coli* K-12, and *L. inocua,* were tested in water, beer, liquid whole egg, apple juice, apple cider, pineapple juice, prune juice. and a model formulated juice. Microwave energy, in the absence of other stresses such as heat, pH, or anti-microbials, did not destroy microorganisms at low temperatures. Results indicated a susceptibility of *Pediococcus* spp. to malic acid in apple juice. This susceptibility was accelerated by a thermal effect at 40°C within the microwave process. Through a contract with the Princeton Plasma Physics Lab, we have modified the RF unit to accommodate double pipe cooling as used in the microwave. We will use the unit to test for non-thermal effects of RF on liquid foods.

IMPACT/TECH TRANSFER B: If and when we prove a non-thermal effect, we will develop a process using appropriate organisms, such as molds and yeast in beer and an *E. coli* analog in cider. Exploit the process by applying it to liquid egg (whole egg, yolk, and egg white) using a *Salmonella* analog.

PUBLICATIONS:

Annous, B., and M. Kozempel. 1998. Influence of growth medium on thermal resistance of *Pediococcus sp.* NRRL B-2354 (formerly, *Micrococcus freudenreichii*) in liquid foods. J. Food Protect. 61(5):578-581.

Kozempel, M., B. Annous, R. Cook, O.J. Scullen and R.C. Whiting. 1998. Inactivation of bacteria with microwaves at reduced temperatures. J. Food Protect. 61(5):582-585.

Kozempel, M., A.M. McAloon and W. Yee. 1998. The cost of pasteurizing apple cider. Food Technol. 52(1):50-52.

Kozempel, M., O.J. Scullen, R. Cook and R.C. Whiting. 1997. Preliminary investigation using a batch flow process to determine bacteria destruction by microwave energy at low temperature. Lebensm.-Wiss. u.-Technol. 30(7):691-696.

Kozempel, M., B. Annous, R. Cook, O.J. Scullen and R.C. Whiting. 1998. Inactivation of bacteria with microwaves at reduced temperatures. J. Food Protect. 61(5):582-585.

Kozempel, M., A.M. McAloon and W. Yee. 1998. The cost of pasteurizing apple cider. Food Technol. 52(1):50-52.

ABSTRACTS:

Kozempel, M., N. Goldberg, A.I. Morgan and E.R. Radewonuk. Combined process of vacuum and steam to "cold" pasteurize poultry. Proc. R.&D.A. October 29, 1997.

PATENTS:

Morgan, A.I. and N. Goldberg. Design of In-line Surface Pasteurizer. U.S.Patent 5,281,451.

Morgan, A.I. "Method of Treating Raw Meat". Canadian Patent 2,116,838.

RAPID DETECTION OF PATHOGENIC BACTERIA IN FOODS BY BIOSENSORS

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OBJECTIVE A: To develop sensitive, specific and rapid processes that require minimal culture enrichment for the detection of pathogenic bacteria in food systems and to compare with traditional microbial detection and sampling methods. Optimize the immunomagnetic-electrochemiluminescent (IM-EC) detection of *E. coli* O157:H7 for practical applications.

PROGRESS A: We have developed an assay for *E. coli* O157 capable of detecting 1 CFU per 25 grams of ground or unground beef in less than 8 hours. Meat samples are enriched in Modified EC medium containing Novobiocin for 5-6 hours, then anti-*E. coli* O157 antibody coated magnetic beads and ruthenium-labeled anti-*E. coli* antibody are added to 1 ml of the enriched but filtered samples. The sample is incubated 1 hour, then analyzed on an ORIGEN instrument obtained from IGEN Int'l. A positive test provides an antibody-based determination of presumptive contamination by *E. coli* O157:H7; if the test is negative, the sample is presumed contamination free. We have also developed H7 DNA primer pairs, one labeled with biotin and the other with ruthenium and have developed a PCR amplification and IM-EC analysis to confirm the presence of *E. coli* O157:H7. The incorporation of DNA-related IM-EC detection to the ORIGEN instrumentation is the goal of our research in the coming year.

IMPACT/TECH TRANSFER A: We have initiated a CRADA with the IGEN Int'l and Warren Analytical Laboratories, the analytical laboratory of ConAgra, to compare this method to the analytical method presently used by Warren. In conjunction with IGEN, we have installed an instrument in the analytical laboratory of a Philadelphia area meat processor to obtain preliminary information before the rigorous test with Warren. We have met with FSIS Special Project and Outbreaks Laboratory personnel and are preparing a proposal to transfer this technology to FSIS.

OBJECTIVE B: Develop a bacterial ice nucleation method for detecting low levels of live *Salmonella* in chicken carcass wash.

PROGRESS B: We (collaboration with IDEXX Laboratories) have described the application of a bacterial ice nucleation detection (BIND) assay for the rapid detection of *Salmonella* in TBS. The BIND assay entails specifically infecting *Salmonella* cells with a bacteriophage which has been genetically engineered to contain DNA that encodes for an ice nucleation protein (INP). Upon infection, *de novo* synthesis of INP in live *Salmonella* spp. occurs and results in the incorporation of INPs into the outer membrane of the organism. Upon supercooling (- 9.3°C) wells containing solutions of *Salmonella* freeze resulting in a color change due to the presence of a phase-sensitive dye. Using this technique and appropriate probability-based protocol modification (i.e., essentially a modified MPN treatment) quantitative detection of cells was achieved with a minimum detectable level (MDL) of approximately 2 *S. enteriditis* per ml in buffer within ca. 3 hr. The MDL for *S. typhimurium* DT104 and *S. abaetetuba* was approximately 6 and 15 cells per ml, respectively. However, the presence of other materials, especially non-pathogenic bacteria with naturally-occurring INPs, in chicken carcass washes seriously compromises detection by yielding false positives. To negate this complication, the bacteria should first be isolated from other contaminants. Thus, we intend to use immunomagnetic beads (IMB) coated with anti-*Salmonella* antibody to capture the bacteria in the wash buffer. We have found that these IMB do not, alone, cause false positives (even at high concentrations) and that IMB-captured bacteria were readily detectable with the BIND method at low *Salmonella* levels (ca. 3 *S. enteriditis* per ml in buffer). In future work we propose to develop a rapid protocol for using IMB in conjunction with the BIND assay for chicken carcass wash buffers. We also propose to use the BIND assay to study capture efficiency of IMB under different conditions at low (e.g., $<$ 100 CFU per ml) bacterial concentrations. This latter work is of interest because all capture efficiency research reported previously in the biosensor literature has utilized unrealistic bacterial concentrations (e.g., $\geq$ 10,000 CFU per ml).

IMPACT/TECH TRANSFER B: The described BIND assay for *Salmonella* was developed in collaboration with an IDEXX scientist. The information was transferred to IDEXX as the research proceeded.

OBJECTIVE C: Improve the specificity of ATP-bioluminescence test for viable cells.

PROGRESS C: Bioluminescence measurement of ATP by luciferin-luciferase has long been used to qualitatively detect the presence of viable cells. By the use of selective lysis reagents, the ATP of somatic and microbial cells may be separately detected. Thus, the method has been utilized for total microbial detection in beef and chicken. However, the application of this bioluminescence method for detecting specific bacteria is yet to be developed. We have utilized IMB coated with anti-*E. coli* O157 antibody to capture *E. coli* O157:H7 in various solutions. The ATP level of captured bacteria were then determined by the luciferin/luciferase assay. Using

oxygen consumption as a measure, we found that the presence of glucose could increase the ATP level of nutrient-deficient but still viable cells. The presence of membrane protonophores, e.g., carbonyl cyanide chlorophenyl hydrazone (CCCP), substantially decreased the ATP levels of viable cells. The effects of glucose and CCCP were not observed in the heat-killed *E. coli* O157:H7. The described approach of using the IMB, glucose and CCCP was able to detect 1 CFU of the bacteria per gram of ground beef after a 6-hour enrichment in the EC medium described in **A**. In the coming year, we plan to work together with Contamination Sciences Inc. to optimize the measuring conditions for practical applications. We also plan to expand the above approach to the measurement of *Campylobacte*r spp.

IMPACT/TECH TRANSFER C: We have established a collaborative arragement with the Contamination Sciences, a company which specializes in bioluminescence measurement, microbead technology and antibody production. Specifically, the company is in the process of developing a CRADA with us to incorporate our IMB approach to develop a more versatile and automated instrument for commercial applications.

OBJECTIVE D: Improve the reproducibility and sensitivity of the automated filtration capture immunoelectrochemical detection system.

PROGRESS D: We previously reported the development of an automated apparatus for detection of bacteria utilizing an integrated filter/electrode unit to capture and detect cells labeled with an alkaline phosphatase-antibody conjugate. This approach provided sensitive detection, but exhibited poor reproducibility due to the pulsed-potential used for detection and fouling of the electrode by the sample. A separate detection cell was developed and coupled to the system. This detector could be operated at a fixed potential and was not exposed directly to samples. In tests with *E. coli* O157:H7, the detection limit was reduced from 10,000 to less than 1,000 cells/ml. The goal of future work will be to develop processing protocols and optimized conditions for assay of *Salmonella* spp. and *E. coli* O157:H7 in food samples.

IMPACT/TECH TRANSFER D: The developed membrane filtration immunomagnetic assay procedure for *E. coli* O157:H7 detection in buffer solution was recently adopted by researchers at the Jefferson Medical University to detect the bacteria in water and urine samples.

OBJECTIVE E: Developed an electromagnetic separator to rapidly concentrate IMB-captured bacteria in solutions.

PROGRESS E: Efficient capture of bacteria on IMB requires bead concentrations of ca. 10^7 per ml. Isolation and concentration of bacteria by batch reactions with IMB followed by magnetic separation is very effective for sample volumes of 1 ml or less. For larger sample volumes batch

reaction is not feasible due to the cost of the IMB and the difficulty in detecting bacteria among the large number of IMB. A solution to this problem is to flow the sample through a bed of retained IMB. If the bed volume is small, then a high IMB concentration can be maintained while using a relatively small number of beads. After capture, the IMB and bacteria can be removed from the bed. We have developed a low-volume flow-through device which retains IMB reversibly in an electromagnet. With the magnetic field on, a sample can be pumped through the device to capture bacteria on the beads. With the magnetic field off, the beads and bacteria can be eluted in a small volume of buffer. Optimum conditions for loading, retaining, and eluting magnetic particles have been determined. Sample can be pumped at up to 5 ml per minute through the device, and the beads can be eluted in less than one ml of buffer. Complete elution of particles required careful demagnetization of the capture cell using a series of current pulses. Goals for the next year will be to optimize conditions for bacterial capture and couple the separator to suitable assays for detection of captured pathogens, e.g., *E. coli* O157:H7 and *Campylobacter* spp.

IMPACT/TECH TRANSFER E: None.

PUBLICATIONS:

Brewster, J.D., and R.S. Mazenko. 1998. Filtration capture and immunoelectrochemical detection for rapid assay of *Escherichia coli* O157:H7. J. Immunol. Meth. (in press)

Fratamico, P.F., and C.G. Crawford. 1998. *Escherichia coli* O157: Detection by commercial immunomagnetic particle-based assays. Encyclopedia Food Microbiol. (in press)

Gehring, A.G., E. Ehrenfeld, J.S. Fanelli, S-I. Tu and P.L. Irwin. 1998 Limit of detection for *Salmonella spp.* using the bacteriophage-induced ice nucleation assay. J. Food Protect. (submitted)

Gehring, A.G., D.L. Patterson and S-I. Tu. 1998. Use of a light-addressable potentiometric sensor for the detection of *Escherichia coli* O157:H7. Anal. Biochem. 258: 293-298.

Irwin, P.L., J.N. Brouillette, A.J. Giampa, K.B. Hicks, A.G. Gehring and S-I. Tu. 1999. Hydration enthalpy and the solvent entropy (hydrophobic) effect. Carbohyd. Res. (submitted)

Mazenko, R.S., F. Riders and J.D. Brewster. 1999. Filtration capture immunoassay for bacteria: optimization and application to urine analysis. J. Microbiol. Meth. (submitted)

Tu, S-I., D.L. Patterson and J. Uknalis. 1998. Adenosine triphosphate enhances the fluorescence of 4',6-diamidino-2-phenylindole (DAPI)-labeled *Escherichia coli* O157:H7. J. Food Safety 18:113-126.

Tu, S-I., D.L. Patterson, J. Uknalis and P.L. Irwin. 1999. Detection of *Escherichia coli* O157:H7 using immunomagneic capture and luciferin-luciferase ATP measurement. J. Food Safety (submitted)

Tu, S-I, J. Uknalis and A.G. Gehring. 1999. Detection of immunomagnetic bead captured *Escherichia coli* O157:H7 by light addressable potentiometric sensor. J. Food Safety (submitted)

Tu, S-I., J. Uknalis, D.L. Patterson and A.G. Gehring. 1998. Detection of immunomagnetically captured, 4',6-diamidino-2-phenylindole (DAPI)-labeled *Escherichia coli* O157:H7 by fluorescent microscope imaging. J. Rapid Meth. Auto. Microbiol. (in press)

NEW TECHNOLOGIES TO IMPROVE AND ASSESS MEAT QUALITY AND SAFETY

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CRIS NUMBER: 1265-41420-001
FSIS CODE: B3MI03
CRIS TERMINATION DATE: February, 2002

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OBJECTIVE A: Determine the effect of hydrodynamic pressure on retail stability and microbial reduction in beef.

PROGRESS A: As a result of major structural alterations in the protein (myofibrillar) structure of meat treated with hydrodynamic pressure (Solomon et al., 1997; Zuckerman and Solomon, 1998) we considered the potential benefits hydrodynamic pressure may have on reducing or eliminating microorganisms associated with meat. Boneless beef strip loins and top rounds were vacuum packaged and treated with the Hydrodyne process (350 g of explosive, 46-cm from bottom of 1060-liter capacity steel container). A portion of each meat cut was removed and re-packaged for a retail display study. Samples were taken at three different periods after treatment: day 7, 17 and 21 for the stip loins and day 10, 17, and 21 for the top rounds. Analysis of pH, purge, thiobarbituric acid-reactive substances (TBARS), aerobic plate count (APC) and anaerobic plate count (AnPC) were performed for each sampling period. Panel discoloration scores and Hunter colorimeter values were obtained each day of the retail display period for both cuts. Preliminary studies on beef (227 g) samples treated with hydrodynamic pressure in plastic 208-liter containers using 100 g of explosive at 38 cm from the bottom of the container suggested successful microbial reduction.

IMPACT/TECH TRANSFER A: Microbial numbers (APC) were low (2.66 and 2.59 $\log_{10}$, cfu for strip loins and top rounds, respectively) at 7 days postmortem in the control samples and increased to 3.45 and 3.47 cfu, respectively by day 21 of refrigerated, vacuum packaged storage. Hydrodynamic pressure treatment resulted in an initial 16% (strip loins) and 12% (top rounds) reduction in APC at 7 days postmortem and 16% (strip loins) and 24% (top rounds) reduction after 21 days of storage. All TBARS (a measure of rancidity) were below 0.4. This is well below 1.0, the point at which rancidity is usually detectable. Extended retail display after an extended storage period increased the amount of TBARS. However, no differences among treatments were revealed for either cut. There was a trend for hydrodynamic pressure treated strip loins to have lower TBARS readings after the extended

retail display. Thus, it appears that the hydrodynamic pressure does not affect rancidity, which implies flavor stability and also may extend shelf life of meat products by reducing microbial spoilage.

OBJECTIVE B: Effect of hydrodynamic pressure on the viability of *Trichinella spiralis* in pork.

PROGRESS B: Pigs of mixed sex and weighing 25 to 40 kg were inoculated via oral gavage with gelatin capsules containing a *T. spiralis*-meat mixture in order to perform three different experiments. A section of muscle was removed from the central portion of the loin from both right and left carcass sides. Loin samples were randomly allocated to hydrodynamic pressure treatment or control groups in each experiment. The first experiment was performed in 208-liter plastic containers with a diameter of 51 cm using 100 g of explosive at 38 cm from bottom. The second and third experiments were performed in a 1060-liter steel container with a 122 cm diameter. In experiment II 150 g of explosive at 61 cm was used and for experiment III 200 g at 38 cm was used.

IMPACT/TECH TRANSFER B: Numbers of *T. spiralis* recovered from infected pork were significantly reduced by hydrodynamic pressure treatment, as compared with untreated, infected pork. However, treatment with these hydrodynamic pressure fronts did not eliminate the infectivity of this parisite when the larvae from the hydrodynamic pressure treated meat were inoculated into mice.

OBJECTIVE C: Identify mechanisms and develop control procedures to prevent inconsistent cooked color/cooked meat temperature relationships.

PROGRESS C: Premature brown color in cooked beef patties at less than safe temperatures presents a potential food safety risk. To determine the prevalence of this situation, a nationwide sampling was conducted by two ARS-USDA (Beltsville, Athens) and three FSIS-USDA (Athens, Alameda, St. Louis) laboratories. Ground beef (476 samples) was selected daily from retail stores over a four-week period in the cities where laboratories were located and in five other distant cities. Ground beef was processed into 112-116 g patties and either cooked immediately or frozen and thawed by microwave, room temperature (two hr) or at 5°C (four hr) before cooking. Additional packages were frozen as bulk product and stored for 7 or 28 days before thawing at 5° C for 18 hr and processing into patties. Patties were cooked on electric griddles to either 57.2, 65.6, 71.1 or 79.4° C before visual and instrumental color evaluations. Using a criterion of complete absence of pink color to be assessed brown, 3.2% of the patties cooked to 57.2°C and 10.2% of the patties cooked to 65.6°C were assessed as brown. Permitting a slight area of pink in patties to also be included as brown resulted in 7.5 and 20.6%

of the patties to be classified a brown when cooked to 57.2 and 65.65° C respectively. Correspondingly, 47.4% and 16.5% of the evaluations were pink or red for patties cooked to 71.1 and 79.4°C respectively. Thawed bulk ground beef produced more brown color when cooked than patties cooked fresh or rapidly thawed. Some general observations from the study were as follows: (1) The Kansas State Color Guide did not always reflect color situations observed in patties from this study, (2) color in cooked patties changes very quickly and is often not equally distributed within a patty, (3) in raw ground beef, considerable variation can exist between product surface and product interior and (4) thawing conditions were often too short to fully thaw product. This study provides evidence that cooked beef patty color is not a good indicator of internal patty temperature.

Variation in internal temperature can also exist within patties during cooking. Infrared thermography was employed in several studies to document this variation using all-beef and ingredient-added patties differing in fat content and cooked from the frozen or thawed state to either 71 or 81-85° C. Thermography revealed temperature ranges within patties greater than 22° C for many patties. Endpoint temperature, product state when cooked, fat content and added ingredients exerted minimal influence on thermal profiles. Infrared thermal imagery revealed the occurrence of considerable temperature variability in cooked beef patties.

Additional studies were conducted to evaluate the influence of outdoor gas grilling, and time post cooking of evaluation on internal temperature and incidence of premature brown color in cooked patties. Removal of patties from grills using visual observation for brown color in a perimeter slit (made with a knife) resulted in a mean temperature of removal of 63.1° C, some pink/red color in the patty interior and a darker red color when observed outside compared with inside controlled lighting. In contrast, when pink color exists in patties cooked on electric griddles, it is often in the outer edge. Over a 75 sec holding period after cooking, more brown cooked color developed. Uncooked color changes from dark red to light pink. For patties removed from griddles at 57.2° C, temperature continues to raise 6.2°C over a 1 min, 38 sec period. For patties removed griddles at 65.6°C, temperature continued to raise 3.8°C over a 1 min, 17 sec period.

IMPACT/TECH TRANSFER C: Results from the joint ARS-FSIS study on premature brown color were presented at a public meeting held in Washington, D.C. in May, 1998. These results were also presented in July, 1998, at the American Meat Science Association annual meeting. From these results and others, FSIS developed a new consumer message that was presented at a press conference in Washington, D.C. on August 11, 1998. The message essentially is that "the only way to determine that a ground beef patty has been cooked to a high enough temperature to destroy harmful bacteria is to use a thermometer. Consumers should not eat ground beef patties that are pink or red in the middle unless a food thermometer has been used to verify cooked temperature." In conjunction with FNS and the National Cattlemen's Beef

Association, questionnaires have been directed to the nation's school food service systems to document the prevalence and causes of persistent pink color in beef patties cooked to 71.1° C.

PUBLICATIONS:

Berry, B.W. 1998. Color of cooked beef patties as influenced by formulation and final internal temperature. Food Res. Int. 30: 473-478.

Berry, B.W. 1998. Cooked color in high pH beef patties as influenced by fat content and cooking from the frozen or thawed state. J. Food Sci. (in press).

Berry, B.W., M.B. Solomon, H. Zuckerman, J.S. Eastridge and J.B. Long. 1998. Application of Hydrodyne technology for military meat products. Proc. R&D Assoc. Mil. Food Pack. Meet. 50 : 279-284.

Gamble, H.R., M.B. Solomon and J.B. Long. 1998. Effects of hydrodynamic pressure on the viability of *Trichinella spiralis* in pork. J. Food Protect. 61:637-639.

O'Rourke, B.M., C.R. Calkins, R.T. Rosario, M.B. Solomon and J.B. Long. 1997. Hydrodyne-treated beef: tenderness, rancidity, and microbial growth. Proc. Int. Cong. Meat Sci. Technol., Auckland, New Zealand, pp. 85-88.

Solomon, M.B., J.S. Eastridge, H.Zuckerman, J.B. Long and W. Johnson. 1997. Hydrodyne treated beef: tenderness and muscle ultrastructure. Proc. Int. Cong. Meat Sci. Technol., Auckland, New Zealand, pp. 89-92.

Zuckerman, H., and M.B. Solomon. 1998. Ultrastructural changes in bovine longissimus muscle caused by the hydrodyne process. J. Muscle Foods (in press).

ABSTRACTS:

Berry, B.W., B.G. Lyon, D.L. Soderberg, T. Phillippo, R. Bakowski, C. Henry, J. Dreas, S. Powell, E. Agamata, C. Yee, N. Clinch and D. Vanlonkhuyzen. 1998. Visual and instrumental evaluations of color in beef patties obtained nationwide and cooked to four internal temperatures. Proc. Recip. Meat Conf. 51:182.

Berry, B.W., D.L. Soderberg and M.E. Bigner. 1998. Thermography profiles of cooked beef patties. Proc. Recip. Meat Conf. 51:182

POSTMORTEM MUSCLE/MEAT CHANGES THAT AFFECT PRODUCT SAFETY AND QUALITY

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CRIS NUMBER: 6612-41420-005
FSIS NUMBER: R3C101
CRIS TERMINATION DATE: June, 1999

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OBJECTIVE A: Validate use of the pink-juice test as a means to determine attainment of end-point temperature (EPT) of 79.4°C in cooked, imported beef to assure inactivation of Foot and Mouth Disease virus; develop an object method to determine this EPT as an alternative to the pink-juice test.

PROGRESS A: This research has been concluded. Results have been reviewed by peers in ARS and FSIS and recommended for publication in a refereed journal. The pink-juice test currently used as the official method to determine the required EPT for imported beef was validated. An alternative method that is rapid and user-friendly was developed and can be used to assess and validate inspector judgements with minimal use of laboratory equipment. Animal age and tissue type were nonsignificant on residual color of expressed juices. A residual pink color remained in the juices at EPT of 79.4°C, therefore, absence of color indicates attainment of this EPT. Residual glutamic-oxaloacetic transaminase (GOT) activity in juices at this EPT is essentially zero; analysis for this enzyme activity is indicative of heat treatment received.

IMPACT/TECH TRANSFER A: These results will allow continued use of the pink-juice test by FSIS inspectors to evaluate EPT of cooked beef imported from countries where Foot and Mouth Disease is prevalent. This test is desirable because it is rapid and has been used for many years. Use of the assay developed to measure residual GOT activity can be used as an alternative to the pink-juice test when an objective method is desired to verify inspector decisions.

OBJECTIVE B: Accurately assess the end-point temperature to which cooked beef imported into the U.S. has been heated by use of multiple estimators.

PROGRESS B: Due to retirement of Support Scientist, progress has been limited to further analysis of previously gathered data. Multiple estimator models using chemical and physical properties of juice mechanically expressed from beef samples which were cooked to known end-point temperatures were evaluated as potential analytical methods. Yellowness (b* values) and glutamic oxaloacetic transaminase (GOT) were found to provide the best estimates with linear models. These models accounted for 83.4% of the variation in EPT when tested between 76 and 84 C. Data gathered from juice samples as opposed to samples from the meat does not appear to improve the precision of EPT estimates.

IMPACT/TECH TRANSFER B: As noted last year, estimates of EPT can be improved by including multiple independent variables in the model. Use of juice samples did not improve the precision.

OBJECTIVE C: Develop and test miniaturized instrumentation for use in sampling and analyzing headspace volatiles of packaged/stored poultry and other meat products for compounds that are indicative of microbial and oxidative spoilage.

PROGRESS C: A CRADA is now active with Perkin-Elmer (PE), Photovac to test hand-held instruments that were recently developed to analyze for total volatile organic compounds in atmospheres. Improvements have been made in the sensing mechanism such that the instrument is now sensitive to specific compounds in ppb quantities. A research program has been designed to test these instruments and further modifications against mass spectral and other methods for headspace analysis of volatiles from chicken pieces stored at 4 and 13°C vs microbial analysis for APC, coliform, *E. coli* and *Salmonella*.

IMPACT/TECH TRANSFER C: This research is consistent with present emphasis of ARS for interaction with industry to develop new technologies relating to food safety. Interaction with PE Photovac provides access to R&D of instrumentation that is not possible within ARS; furthermore, interaction with ARS provides PE Photovac with access to commodities, laboratory facilities and research capabilities that are not otherwise available to them.

OBJECTIVE D: Evaluate use of High Performance Liquid Chromatography (HPLC) for characterizing advanced meat recovery (AMR) product from beef sources.

PROGRESS D: Initial tests have been run using DEAE Ion Exchange chromatography with photodiode array detection to characterize advanced meat recovery (AMR) product from beef sources. AMR sources included product made from beef neck bones (NK), beef neck bones and ribs combined (NKRB), hand-trimmed neck bones (NKHDTRM), ground beef (GB), and ground beef with 10% AMR (GB10AMR). Separation profiles at 545nm (characteristic for

the oxy-hemoglobin and myoglobin protein forms) showed NK AMR with 2 principal peaks at 5.4 and 7.5 min compared to NKHDTRM having only 1 peak at 5.4 min. NKRB AMR had the 5.4 min component and a second principal peak at 6.3 min with 2 minor peaks at 7.8 and 8.5min. GB and GB10AMR had two characteristic peaks at 4.3 and 5.6 min. These two peaks had inverse ratios to each other within products.

IMPACT/TECH TRANSFER D: This method has potential for FSIS to use as a laboratory method to characterize and determine the presence of advanced meat recovery product.

ABSTRACTS:

Davis, C.E. 1998. Fluorometric determination of acid phosphatase in cooked, boneless, non-breaded broiler breast and thigh meat. PVM 3:1997. J. AOAC Int. 81:4.

Davis, C.E., W.R. Windham and B.G. Lyon. 1998. Acid phosphatase activity and myoglobin denaturation as end-point temperature indicators in cooked ground beef patties. Annu. IAMFES Meet.

Windham, W.R., C.E. Davis and B.G. Lyon. 1998. Determination of endpoint temperature in cooked groung beef patties by Near Infrared Reflectance Spectroscopy. Annu. IAMFES Meet.

Senter, S.D., and J.W. Arnold. 1998. Detection of chicken breast meat bacterial spoilage using photoionization detection, GC-MS analysis and concomitant microbial analysis. Annu. Poultry Sci. Meet.

NIR SPECTROMETRY TO MEASURE NUTRIENTS

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FSIS CODE: R1CI01
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OBJECTIVE A: Determination of end point temperature (EPT) in cooked ground beef by near infrared reflectance (NIR) spectroscopy.

PROGRESS A: NIR is under study in cooperation with the Poultry Meat Processing and Meat Quality Research Unit, Richard B. Russell Agricultural Research Center, and FSIS Eastern Laboratory to determine if it can be used to determine EPT in cooked ground beef patties. Meat juices, after cooking patties to an EPT of 135°, 150°, 165°, and 175° F, were blotted on 3.7 cm glass micro fiber filters and dried in forced-air oven at 50°C for 15 minutes. Near infrared spectra of the dried micro fiber filters were collected from 400 to 2500 nm. Near infrared spectra (780-2498nm) and visible spectra (400-780nm) were related to EPT. The error for determining EPT by NIR was 2.8° F. Regression analysis using visible absorbance gave the poorest prediction results possible due to modeling the myoglobin oxidative state variation. Near infrared spectra showed a significant difference in protein and color absorbance with increased EPT. Studies are ongoing to determine single or a combination of NIR wavelengths to predict EPT.

IMPACT/TECH TRANSFER A: These preliminary results show that this 15-min test could have potential as an EPT indicator for verifying FSIS/FDA requirements for fully cooked hamburger patties. In addition, the method with a single or combination of wavelengths could be used in fast food quality assurance programs to verify compliance with FSIS/FDA regulations.

OBJECTIVE B: Establish valid laboratory methods for assaying iron as an indicator of soft bone constituents in trim beef derived from advanced meat recovery systems (AMRS) and to compare hand trim beef to AMRS beef.

PROGRESS B: AMR trim beef samples were analyzed by a dry ash, and two wet ash iron procedures. The wet ash procedures used hydrochloric acid to oxidize the organic matter and a mixture of nitric and sulfuric. The mean iron content was 2.79, 4.91 and 4.13mg/100g for wet HCL, dry ash, and wet nitric/sulfuric, respectively. The wet nitric/ sulfuric method used 33% nitric acid rather than concentrated nitric, resulting in a lower mean value due to incomplete ashing. An additional random set of AMR beef were analyzed for iron using a wet ash procedure with concentrated nitric/sulfuric mixture and a dry ash method. The mean iron content was 6.08 and 5.61mg/100g for the wet and dry ash methods, respectively. The standard error of a difference due to method was 0.63mg/100g. The within method standard error of the lab was 0.42 and 0.38mg/100g for the wet and dry ash methods, respectively. From our evaluation of these methods, we selected the dry ash method for routine analysis. To determine within and among laboratory variability, three collaborating laboratories analyzed 5 AMR samples for iron using the dry ash method. The laboratories were ARS, University of Wyoming, and Rock River Laboratory. The within-laboratory repeatability (S_r) and between-laboratory reproducibility (S_R) was 0.10 mg/100g and 0.57 mg/100g, respectively.

IMPACT/TECH TRANSFER B: These results indicate that iron content of AMR trim beef determined with a dry ash or wet ash (nitric/sulfuric) procedure can be used by FSIS as a performance standard for soft bone constituents. However, ARMS trim beef may pass or fail a performance standard due to iron methodology and analytical variability.

PUBLICATIONS:

Windham, W.R. and W.H. Morrison. 1998. Prediction of fatty acid content in beef neck lean by near-infrared reflectance analysis. J. Near Infrared Spectroscopy 5:(in press).

ABSTRACTS:

Windham, W.R., C.E. Davis and B.G. Lyon. 1998. Determination of end point temperature in cooked ground beef patties by near infrared reflectance spectroscopy. Annu. IAMFES Meet., 19.

Davis, C.E., W.R. Windham and B.G. Lyon. 1998. Acid phosphatase activity and myoglobin denaturation as end-point temperature indicators in cooked ground beef patties. Annu. IAMFES Meet. 19.

Windham, W.R. 1998. Scientific studies on AMR beef trim produced by industry. ABC Res. Corp. Annu. Tech. Symp. Gainesville, FL.

Part IV. RESIDUE DETECTION AND CHEMICAL ANALYSIS

ADVANCED TECHNOLOGIES FOR THE ANALYSIS OF CONTAMINANTS IN MEAT, POULTRY AND EGGS

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FSIS CODE: E2CC03
CRIS TERMINATION DATE: September, 2000

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OBJECTIVE A: Develop supercritical fluid extraction (SFE) methods using carbon dioxide for the isolation of sulfonamides (SAs) from meat tissues and eggs at the regulatory tolerance level.

PROGRESS A: To broaden the applicability of the SFE method for chicken liver tissue from sulfamethazine, sulfa-dimethoxine and sulfaquinoxaline, four additional SAs were added to the analytical scheme. These included: sulfa-chloropyridazine, sulfamerazine, sulfapyridine and sulfathiazole. Experiments were carried out on determining the effect of temperature on SA solubility range 40-100^{0}C. The highest recoveries were obtained for five of the seven SAs at 60^{0}C. Acetonitrile modified CO_2 was then examined as means to improve the recovery of these more polar SAs. The use of 10% acetonitrile modified SC-CO_2 at 680 atm. and 40^{0}C was effective in recovering (>70%) six of the seven SAs in chicken livers fortified at the 100 ppb level. Due to its poor solubility in SC-CO_2, only 30% recovery was obtained for sulfathiazole, the most polar sulfonamide.

IMPACT/TECH TRANSFER A: This is the first report of an SFE method applied to multiple sulfonamide residues and the first report of applying SC-CO_2 to effectively extract an incurred SA from animal tissue. The method will be transferred to FSIS and FDA for evaluation. The same laboratories carrying out SFE for SAs on meat tissue will now be able to perform these analyses on eggs.

OBJECTIVE B: To apply SFE for the extraction of chloramphenicol drug residues in tissue and eggs.

PROGRESS B: Studies were initiated on the SFE of chloramphenicol (CAP) in whole eggs fortified at the 10 ppb level, without the use of a solvent modifier. Here, CAP was trapped in-line on Florisil, and after elution, analyzed by HPLC-UV detection at 280 nm. Using unmodified CO_2 and SFE conditions of 680 atm. and 80^0C, recoveries of ≥80% were obtained. Eggs containing incurred CAP were obtained by feeding laying hens a single dose of this drug over a two days. Eggs collected over 12 days were analyzed by SFE (range n.d.- 174.5 ppb, mean 60.5 ppb). The SFE values compare favorably to the results obtained by a solvent extraction method (mean of 60.5 ppb). SFE studies were also carried out on the isolation on CAP from animal tissues such as chicken muscle, liver and pork kidney fat. The analyte was trapped on an in-line Florisil trap also using unmodified CO_2 as the extracting solvent at 680 bar and 80^0C. Recoveries in the range of 55% were lower than those recorded for eggs due the more retentive nature of the animal matrices.

IMPACT/TECH TRANSFER B: Drugs in this class are prohibited from use in food-producing animals; however their illegal use to combat serious infections still occurs. A screening method for this potentially toxic compound will allow the regulatory agencies to more effectively screen for this drug.

OBJECTIVE C: Apply SFE technology to the isolation of dioxins from animal-derived products.

PROGRESS C: Because of the successful application of SFE for organochlorine pesticides (OCPs) in eggs, this work was extended to include other organochlorine compounds, namely dioxins, at the low ppt level. It was thought that SFE might provide an extract sufficiently clean for GC-electron capture detection (ECD) or GC-MS. Studies were initiated on eggs fortified with tetrachloro- and octachlorodioxins, the latter being the most difficult to extract by SFE. 1,3,7,8-Tetrachlorodioxin was used as a surrogate for the more toxic 2,3,7,8-tetrachloro- derivative. Major problems were encountered with the sensitivity of the GC-ECD detection system along with a high degree of interfering contaminants in all the solvents and sorbents. It was concluded that even with cleaner egg extracts, we had no way of assessing how clean or dirty they were compared to those obtained by the currently used solvent extraction-cleanup regulatory methods where the dioxins are analyzed by GC-MS/MS. Work on eggs was abandoned for the time being to work on simpler matrices having higher levels of incurred dioxins. This approach was thought to be simpler. We would not have to deal with the problem of separating the dioxins from fat in the egg yolk. Efforts were made to isolate dioxins in pentachlorophenol-treated wood, as would be found in a farm environment. These wood samples were obtained from Vern Feil (ARS, Red River Agr. Res. Ctr., Fargo, SD) who determined that ppb levels of incurred dioxins were present, not ppt levels normally found in environmental samples and foods. So much organic material that responded to the

GC-ECD was extracted from the wood that the ability of SFE to isolate dioxins and to simultaneously effect a partial extract cleanup could not be evaluated. The chromatograms went off scale and did not return back to baseline even after a 75 min period. This occurred even using a sample size of 0.1-0.5 g wood. There was also an indication of other sources of contamination that gave peaks in the area where dioxins eluted. Subsequent work focused on the use of SFE to isolate dioxins from ball clay used as an anti-caking agent in chicken and fish feed. Ball clay samples containing high levels of incurred dioxins from the mine responsible for the dioxin-feed incident were obtained from the EPA laboratory at the Stennis Space Center, MS. To date, the SFE extracts obtained are cleaner than those observed from eggs. Work is continuing in this area because SFE appears to be viable for this application.

IMPACT/TECH TRANSFER C: The recent events where dioxins were found in chicken tissue, eggs and catfish show the need for cost-effective, specific laboratory screening procedures. Results from these studies will lead to rapid regulatory implementation of these methods. SFE should also help in the confirmation of these compounds at the ppt level, because the need for extensive extract cleanup currently required may not be needed.

OBJECTIVE D: Apply SFE technology for the isolation of triazine (TRZ) herbicides from eggs.

PROGRESS D: Studies were initiated on the extraction of triazine (TRZ) herbicides from fortified eggs (100 ppb). Preliminary results indicate that atrazine, simazine, propazine, prometryne, simetryn, and terbutylazine were recovered in yields $>70\%$ by SFE with off-line trapping, without the use of a solvent modifier. Gestamine, prometon and secbumeton were $>50\%$. The extraction conditions were: 40^{0}C at 10,000 psi, flow rate 3.0 ml/min for total of 120 L. Other TRZs, i.e., ametryn, cyanazine and prometon at residue levels, will be evaluated for their extractability by SFE so that a multiresidue approach will be possible.

IMPACT/TECH TRANSFER D: Under risk assessment guidelines it is expected that triazine herbicide residues will need to be monitored in animal tissue and eggs. Their continued extensive use on animal feed crops, especially corn, is highly controversial since several members of this class exhibit carcinogenic and immunotoxic activity.

OBJECTIVE E: Investigate the use of automated on-line microdialysis for fluoroquinolone antibiotic residue sample preparation (analyte extraction and concentration) prior to HPLC determination.

PROGRESS E: The isolation of fluorquinolones (FQs), flumequine (FMQ) and oxolinic acid (OXO), from fortified chicken liver was achieved using liquid-liquid extraction, aqueous on-line micro-dialysis, and trace enrichment performed using a Gilson ASTED system. Analysis

of the isolated compounds in the tissue extracts was made using reversed-phase HPLC and single wavelength fluorescence detection. This procedure yielded excellent recoveries at 50 ppb (92%, 9% RSD) and 10 ppb (94%, 10% RSD) spiking levels for FMQ and at the 25 ppb (86%, 5% RSD) and 5 ppb (99%, 6% RSD) spiking levels for OXO. Clean chromatograms were obtained, allowing the easy detection of 10 ppb and 5 ppb for FMQ and OXO, respectively. The method was expanded to include sarafloxacin (SAR) in an attempt to develop a multiresidue method for other members of this class of anti-bacterial agents now approved for use in poultry. Since sarafloxacin has a different fluorescence absorption maximum wavelength than the other two FQs, two HPLC runs are required to obtain the required sensitivity. To solve this problem, a programmable FLD was purchased. It permitted the detection of all three FQs with a single HPLC run. Employing standards, a limit of quantitation of 1.0 ppb and limit of detection of 0.2 ppb, were obtained, limits which could not be attained by a single wavelength FLD.

The effective application of this detector with micro-dialysis for the analysis of SAR, OXO, and FMQ in fortified chicken liver tissue was demonstrated. Recoveries > 85% were obtained over a range of 1-100 ppb. Chicken livers containing incurred SAR were obtained from an FDA, CVM collaborator, who dosed the chickens at two levels for three consecutive days. SAR was isolated by microdialysis and by a solvent extraction procedure, then analyzed under the same HPLC-FLD conditions. The results obtained for the micro-dialysis method were comparable to those obtained by the other procedure. However, much cleaner chromatograms and greater sensitivity were obtained by the microdialysis method suggesting the application of this extraction technique is feasible for other members of this class of water soluble compounds.

The application of on-line microdialysis for the isolation of the FQ, SAR from chicken eggs was demonstrated. Recoveries of SAR of 87-100% were obtained from eggs fortified from 1-100 ppb. Eggs containing incurred SAR were obtained from FDA, CVM. The eggs were harvested over a three-day dosing and a five-day withdrawal period. Incurred SAR values 50-187 ppb with RSDs of 7-13% were obtained. Over 35 egg samples/day could be processed by the on-line microdialysis method requiring less than 2 hr/day of analysis time.

IMPACT/TECH TRANSFER E: The use of microdialysis in combination with a programmable fluorescence HPLC detector for the analysis of three FQs at the ppb level was demonstrated. This, combined with the advantages of using on-line microdialysis with its low organic solvent consumption, short sample processing time, small sample volume, automation and high sample throughput, makes this technique promising for routine monitoring by the regulatory agencies. FDA's CVM has adopted this method and has begun trials within the agency to determine its suitability as an official method for FQs in milk.

PUBLICATIONS:

Cohen, E., R.J. Maxwell and D.J. Donohue. 1998. Automated multi-residue isolation of fluoroquinolone antbacterials from fortified and incurred chicken liver using on-line micro-dialysis and HPLC with programmable detection. J. Chromatog. (in press)

Eng, G.Y., R.J. Maxwell, E.G. Piotrowski and W. Fiddler. 1998. Determination of flumequine and oxolinic acid in fortified chicken tissue using on-line microdialysis and high-performance liquid chromatography with fluorescence detection. J. Chromatog. A., 799:349-354.

Fiddler, W., J.W. Pensabene, R.A. Gates and D.J. Donoghue. 1998. Supercritical fluid extraction of organochlorine pesticides in eggs. J. Agric. Food Chem. (in press)

Maxwell, R.J. and Cohen, E. 1998. Comparison of programmable vs. single wavelength fluorescence detection of three fluoroquinolone antibacterials isolated from fortified chicken liver using coupled on-line microdialysis and HPLC. J. High Res. Chromatog. 21:241-244.

Maxwell, R.J., E. Cohen and D.J. Donohue. 1998. Determination of sarafloxicin residues in fortified and incurred using on-line microdialysis and HPLC/programmable fluorescence detection. J. Agric. Food Chem. (submitted)

Maxwell, R.J., and A.R. Lightfield. 1998. Multiresidue SFE method for the recovery of three sulfonamides from fortified chicken liver at low ppb levels. J. Chromatog. (in press)

Maxwell, R.J., and A.R. Lightfield. 1998. An improved method for efficient predevelopment washing of HPTLC plates. J. Planar Chromatog. (in press)

Maxwell, R.J., O.W. Parks, R.J. Shadwell, A.R. Lightfield, C. Henry and B.S. Fuerst. 1998. Supercritical fluid extraction of melengesterol acetate from bovine fat tissue. *In*: Methods in Molecular Biology: Supercritical Fluid Methods (in press)

O'Keeffe, M.J., M. O'Keeffe, J.D. Glennon, A.R. Lightfield and R.J. Maxwell. 1998. Supercritical fluid extraction of clenbuterol from bovine liver tissue. Analyst. (in press)

Pensabene, J.W., W. Fiddler and D.J. Donohue. 1998. Comparison of supercritical fluid extraction and a solvent method for the determination of incurred sulfamethazine in chicken eggs. J. Food Sci. 63:25-26.

Stolker, A.A.M., L.A. van Ginkel, R.W. Stephany, R.J. Maxwell, O.W. Parks, A.R. Lightfield and M.B. Medina. 1998. Supercritical fluid extraction of Nortestoster-one, Testosterone and Methyltestosterone at low ppb levels from fortified bovine urine. J. Chromatog. (in press).

ABSTRACTS:

Fiddler, W. 1998. Isolation of chemical residues from animal products by supercritical fluid extraction. Southeastern Pennsylvania Chapter Amer. Chem. Soc.

Maxwell, R.J. 1998. Isolation of trace level chemical residues from food products by supercritical fluid extraction. Tri-State Supercritical Fluid Chromatog. Conf. Columbus, OH.

Maxwell, R.J., and A.R. Lightfield. 1998. Effect of varying temperature on the trace level recovery of seven sulfonamides from fortified chicken liver. 8th Int. Symp. Supercritical Fluid Chromatog. Extraction, St. Louis, MO.

Pensabene, J.W., and W. Fiddler. 1998. Isolation of chloramphenicol from whole egg by supercritical fluid extraction. 112th Assoc. Off. Anal. Chem. Annu. Meet., Montreal, Canada.

Reeves, V., E. Cohen and R.J. Maxwell. 1998. Analysis of ciprofloxacin, enrofloxacin, and their desethylene metabolites in bovine plasma and milk by on-line micro-dialysis and liquid chromatography with fluorescence detection. 112th Assoc. Off. Anal. Chem. Annu. Meet., Montreal, Canada.

METHODOLOGY DEVELOPMENT FOR RAPID ANALYSIS OF DRUG AND PESTICIDE RESIDUES IN FOOD ANIMAL PRODUCTS

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OBJECTIVE A: To develop, evaluate, and provide confirmatory testing of highly sensitive, inexpensive, monoclonal antibody-based, immunoassays useful for detection and quantification of chemical residues in animal products and body fluids either on the farm, in the processing plant, or for laboratory-based analyses.

PROGRESS A: Development of new, monoclonal antibody based immunoassays are in progress for a number of compounds, including Halofuginone, fluoroquinolones, 4,4'-dinitrocarbanilide, ceftiofur, tylosin and tilmycosin. These compounds serve as test cases and many have been identified by FSIS as being important for development of rapid immunoassays.

Halofuginone (Hal). The halofuginone immunoassay detects residues in chicken livers in the 50 to 200 ppb range. The assay uses a simplified sample preparation method that eliminates the need for organic chemicals. Thus, this assay demonstrates the usefulness of immunochemistry specifically in the areas of reduced reliance on organic chemicals and improving the efficiency of the assay by simplifying sample preparation and analyte detection. A joint study with an FSIS scientist at the FSIS Midwestern Research Laboratory has been completed and the data submitted for publication in the general scientific literature. Briefly, we have analyzed 496 samples using both the ARS ELISA and an HPLC method conducted at the FSIS Midwestern Laboratory. Both methods gave similar results. Only 5 samples were measured by the ELISA to be greater than 100 ppb but less than 160 ppb and 2 samples fell in this category using the FSIS HPLC method. More importantly, neither method measured any violative samples, i.e., greater that 160 ng/g. Thus, using the ELISA, comparable results, i.e., no violative samples, were obtained in a fraction of the time required to complete the HPLC method with a substantial reduction in generation of organic waste.

Ceftiofur (Cef). Ceftiofur is an FDA-approved veterinary cephalosporin antibiotic for the treatment of respiratory diseases in cattle, horses, and swine. We have developed a simple

immunoassay for detection of ceftiofur and its metabolites in meats and milk. The ARS ELISA detects not only ceftiofur but the major metabolite of ceftiofur, desfurylceftiofur and protein conjugates of desfurylceftiofur. Thus, unlike the HPLC methods which only measure parent or a specific metabolite, the ARS ELISA results should be expressed as ceftiofur equivalents because it measured total residue. Sample preparation for the ARS ELISA is a simple extraction in aqueous buffer for tissue and a simple dilution for milk. Good recovery of analyte was observed in fortification experiments and in incurred residue studies.

Fluoroquinolones (Flu). Fluoroquinolones are antibiotics used to control *E. coli* in poultry. A series of monoclonal antibodies to sarafloxacin have been developed and characterized. Studies aimed at formatting these antibodies into a simple immunoassay for analysis of chicken tissue have been initiated. In addition, studies have been completed describing a rapid ELISA based on these antibodies for fluoroquinolone detection in eggs. Finally, the anti-sarafloxacin antibodies served as a model for development of an automated on-line immunoaffinity-HPLC based assay capable of detecting six different fluoroquinolones (See Objective D).

4,4'-Dinitrocarbanilide (44D). Nicarbazin is a drug comprised of a 1 to 1 molar ratio of 4,4'-dinitrocarbanilide (DNC) and 4,6-dimethyl-2-pyrimidinol that is used to prevent outbreaks of cecal and intestinal coccidiosis in chickens. DNC is the active agent, and it is used by FDA to determine the nicarbazin residues in chicken tissues. Likewise, nicrabazin determination using the established FSIS method measures the levels of DNC. Due to the difficulty of using derivatives of DNC to produce antibodies, we have synthesized a number of mimics of DNC for use as immunogens. These studies have employed sophisticated computer-assisted molecular modeling methods to aid in evaluating the potential of these mimics. These studies have been initiated with a CRADA partner (International Diagnostic System Corp.).

Other Compounds and Activities (OC). We continue a collaboration with Dr. Bruce Hammock, University of California-Davis, to produce anti-dioxin immunoassays and analogs for dioxin. These utilized low-energy molecular models of the polychlorinated dioxins and furans. Likewise, we continued our CRADA efforts with EnviroLogix, Inc., adapting previously developed anti-glycoalkaloid antibodies into a rapid immunoassay for detection and quantification of these toxins in tomatoes and potatoes. These efforts represent a collaborative effort with an ARS scientist at the WRRC and have resulted in submission of a manuscript describing the performance of the commercial immunoassay. A long standing collaboration with Dr. Steve Safe, Texas A&M University, dealing with the function of the dioxin receptor continues.

IMPACT/TECH TRANSFER A: Hal. This immunoassay has been formatted into a simple immunoassay package and was previously transferred to FSIS scientists at the Midwestern Research Laboratory. The economic impact of immunochemical methods is clearly demonstrated by this assay. Analysis of the samples by the traditional HPLC method are estimated to require up to 6 man-months versus less than 1 man-month for the ARS ELISA. This assay serves as an example of the potential application of immunochemistry in residue analysis. The simplicity of the sample extraction and detection steps allow this assay and other similar immunoassays to be utilized by less trained individuals on site at the packing house eliminating the expense associated with sample transport to a centralized laboratory for an instrumental analysis.

Cef. A patent covering the anti-ceftiofur monoclonal antibody has been allowed by the U.S. Patent Office and will issue in its final form by the end of 1998. In addition, the antibody has been licensed to a private kit manufacturer and is an integral part of a commercial immunoassay for detecting and measuring beta-lactam antibiotics in milk. Thus, the commercial application of this research already is having a direct and positive impact on food safety.

Flu. Rapid tests such as this one should help producers, as well as government agencies, to screen poultry products for the presence of fluoroquinolone residues.

44D. Upon successful generation of suitable monoclonal antibodies, they will be incorporated into a simple immunoassay by our CRADA partner and be available as a kit for rapid diagnostics of this drug.

OC. Development of rapid tests for analysis of dioxins in foods would be highly desirable since present technology is time consuming and costly (in excess of $1500/sample). A license was granted to our CRADA partner for use of the anti-glycoalkaloid monoclonals and these are now available as a commercial kit for measurement of these toxins in potatoes.

OBJECTIVE B: To develop methodologies for production of monoclonal antibodies using recombinant DNA techniques and cost-effective methods for expression of such recombinant antibodies (rAb) in bacterial, or mammalian cell systems.

PROGRESS B: Recombinant anti-dioxin antibodies (rAB) have been produced by cloning the genes encoding the heavy and light chains of antibody molecules. Briefly, the antibody heavy and light chain genes are cloned and modified to facilitate their insertion into appropriate expression vectors using a modified Polymerase Chain Reaction (PCR) method. The resultant rAB molecules were expressed in bacterial vectors. The advantages of rAB's versus traditional antibodies are numerous and include the ability to genetically alter the rAB molecules to change the specificity and sensitivity of the antibody molecule using simple and rapid recombinant DNA techniques. In addition, rAB methods reduces the reliance on the use of animals for production

of new, novel immunochemical reagents. The recombinant anti-dioxin antibodies are expressed as recombinant FAB fragments in our system. Recombinant antibodies were generated from two different anti-dioxin monoclonals. Genetic manipulation of these rAB have allowed us to increase antibody sensitivity by nearly 5-fold.

IMPACT/TECH TRANSFER B: These molecular studies represent an ongoing effort to improve the methods to produce antibodies and other useful binding proteins that can be used in biological arrays to detect chemical analytes. Such techniques have direct applications in new fields such as in microchip arrays and biosensors.

OBJECTIVE C: The development of multianalyte immunoassays using on-line immunoaffinity-HPLC Microanalytical Workstation.

PROGRESS C: Traditionally, immunoassay methods for residue analysis are viewed as single analyte methods. In reality, most antibodies, both polyclonal and monoclonal, have some degree of cross-reactivity with structurally related drugs and even with some metabolites of the parent drug. In fact, cross-reactivity often is cited as a limitation of immunoassays requiring that results be expressed as drug equivalents versus drug concentration because of the uncertainty of which cross-reacting analyte is responsible for the signal. However, in principle, this inherent cross-reactivity of antibodies, particularly that of a well characterized monoclonal antibody, can be exploited to detect a number of related drugs with a single antibody. Using our anti-fluoroquinolone monoclonal we have demonstrated the ability to generate a multidimensional, multianalyte immunoassay. The assay is performed on an Intergral Microanalytical Workstation (Perkin-Elmer, Inc.) equipped with the ability to utilize multiple binding and separation columns, multiple reagents, and fluorescent detection in an automated mode under computer control. Briefly, sample extracts and/or mixtures of standards are first trapped on an in-line immunoaffinity column. This column is washed and bound material automatically eluted to a reverse phase column for analyte separation. Our initial results demonstrate the ability to quantify up to six fluoroquinolone antibiotics (difloxacin, sarafloxacin, trovafloxacinin, enrofloxacin, norfloxacin, and ciprofloxacin) present in a mixed standard fluoroquinolone library. In fact these fluoroquinolones could be differentially eluted as two groups (high and low affinity binders) on the immunocapture column which facilitated further separation on the reverse phase column. The assay is completed in less than 15 minutes, and the instrument is capable of automatically injecting up to 105 samples. This assay has been applied to analysis of raw milk, and studies with chicken tissues are in progress.

IMPACT/TECH TRANSFER C: The ability to convert single analyte immunoassays to automated multianalyte immunoassays will greatly enhance the application of these methods for residue analysis. In addition, these studies are the first to demonstrate the ability to utilize the

inherent differential affinity of antibody molecules to separate analytes based on their interaction with a single immunoaffinity column. Refinement of these methods may allow development of even more simplified methods using only a single immunoaffinity column to resolve complex mixtures of compounds. These studies have been prepared and submitted for publication in the scientific literature.

OBJECTIVE D: To develop automated immunochemical detection assays for residues and enteric pathogens using an Immunomagnetic Electrochemiluminescence Biosensor.

PROGRESS D: These studies only recently have been initiated but stem from the need to improve the performance of immunoassays, to improve automation, and to reduce the number of steps often associated with traditional immunoassays such as the ELISA. Additionally, use of more sophisticated detectors such as luminescence is possible with instrumental methods resulting in improved assay sensitivity. Improving assay sensitivity will translate into more simplified sample preparation for residue for residue analyses, and increase confidence in assays for detection and identification of bacteria. Presently we have generated 15 independent monoclonal antibodies that bind different *Campylobacter* species. Since any immunoassay, independent of the format, is fundamentally influenced by the characteristics of the antibody-antigen interaction we are evaluating each of these monoclonal antibodies to determine its specificity, and sensitivity when it is used as the capture component in an immunomagnetic electrochemiluminescence assay. We are using a commercially available instrument in these studies. In addition, we have evaluated this instrument using an anti-*Salmonella* monoclonal antibody produced in our laboratory. Finally, using the anti-ceftiofur monoclonals described above, we have demonstrated the application of this instrumental method for detection of residues.

IMPACT/TECH TRANSFER D: The development of sensitive instrumental immunoassays will greatly facilitate the transfer of immunochemical methods into analytical and bacterial laboratories. The application of multipurpose instruments using formats where only the antibody needs to be supplied greatly reduces the reliance on multicomponent immunoassay kits. Such assay formats should reduce the time between antibody development and assay availability.

PUBLICATIONS:

Beier, R.C., T.J. Dutko, S.A. Buckley, M.T. Muldoon, C.K. Holtzapple and L.H. Stanker. 1998. Detection of halofuginone residues in chicken liver tissue by HPLC and a monoclonal-based immunoassay. J. Agric. Food Chem. 46:1049-1054.

Lee, N.J., C.K. Holtzapple and L.H. Stanker. 1998. Cloning, expression, and characterization of recombinant Fab antibodies against dioxin. J. Agri. Food Chem. 46:3381-3388.

Nigg, H.N., and R.C. Beier. 1998. Agrochemicals and their impact on environment and human health, 11 pp. *In:* Proc. Sustainable Agric. Tropic Subtropical Highlands: Special Reference Latin America (SATHLA), Rio de Janeiro, Brazil.

Sanborn, J.R., S.J. Gee, S.D. Gilman, Y. Sugawara, A.D. Jones, J. Rogers, F. Szurdoki, L.H. Stanker, D.W. Stoutamire and B.D. Hammock 1998. Hapten synthesis and antibody development for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) immunoassays. J. Agric. Food Chem. 46:2407-2416.

Smith, B.I., G.A. Donovan, C. Risco, R. Littell, C.R. Young, L.H. Stanker and J. Elliott. 1998. Comparison of various antibiotic treatments in cows diagnosed with toxic puerperal metritis. J. Dairy Sci. 81:1555-1562.

Wilson, C.L., J. Thomsen, D.J. Hoivik, M.T. Wornke, L. Stanker, C. Holtzapple and S. Safe. 1997. Aryl hydrocarbon (Ah) nonresponsiveness in estrogen receptor-negative MDA-MB-231 cells is associated with expression of a variant Arnt protein. Arch. Biochem. Biophys. 346:65-73.

PATENTS:

Stanker, L.H., B. Rose, C. Holtzapple and J. DeLoach. 1998. Monoclonal Antibodies to Ceftiofur and Assays for the Same. U.S. Patent, June 24, 1998.

DEVELOPMENT OF METHODS OF ANALYSIS FOR RESIDUES IN MEAT AND OTHER AGRICULTURAL COMMODITIES

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CRIS TERMINATION DATE: December, 1999

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OBJECTIVE A: Develop quantitative multiresidue methods of analysis for chemical residues in meat and other agricultural products suitable for regulatory purposes, emphasizing the use of gas chromatography (GC), HPLC, ion-trap mass spectrometry (ITD), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), capillary electrophoresis (CE), and other techniques to maximize recoveries of analytes while minimizing or eliminating matrix interferences.

PROGRESS A: Ion-Trap MS Analysis of Meat: A method of analysis for multiple diverse pesticides was developed for fatty samples using extraction with acetonitrile, solid-phase extraction (SPE) clean-up, and gas chromatography/ion trap mass spectrometric detection (GC/ITD). The method is reasonably fast and easy, enables extraction of meat tissue as well as fat, expands the range of pesticides analyzed, uses no chlorinated solvents, and provides a single step quantitation and confirmation analysis. The method was utilized in the analysis of oysters for NOAA's Mussel Watch Project, and analysis of meat was also evaluated. The detection limits for representative organochlorine (OC), organophosphorus (OP), pyrethroid, and other pesticides were below regulatory tolerance/action levels for meat.

FSIS uses an automated approach using gel-permeation chromatography (GPC) for clean-up and GC/electron-capture detection (ECD) for halogenated analytes only. Samples provided by the FSIS Western Lab were analyzed using the GC/ITD, and the range of analytes was expanded, but the detection limits for the OC pesticides were not as low as the GC/ECD obtains. A GPC instrument similar to the model used by FSIS was obtained on loan from the Michigan Dept. of Agriculture, and the appropriate column and other materials were obtained. Studies will be conducted to determine if the small sample size of the current FSIS approach can be increased to lower the detection limit using the GC/ITD approach.

Supercritical Fluid Extraction: The SFE pilot study for the USDA AMS Pesticide Data Program continued in the evaluation of SFE and GC/ITD in the analysis of multiple pesticide residues in fruits and vegetables. In 1998, tomatoes and strawberries were analyzed from the New York Dept. of Agriculture using traditional and SFE approaches. The 1997 pear results showed good correlation between the different methods, with each approach having advantages and disadvantages with respect to the other. The significantly lower costs, ease of use, reduced labor, and many other benefits of the SFE and GC/ITD approach make it the more efficient method overall. The protocol for a collaborative study, "Pesticide Analysis of Nonfatty Foods Using SFE and GC/MS," through the AOAC® Official Methods Program has been submitted, and more than 16 collaborators have agreed to participate.

Matrix Enhancement Effect: A study of the matrix enhancement effect in GC analysis of OP pesticides was conducted in collaboration with Frank Schenck of the FDA. The matrix enhancement effect is caused by the interactions of pesticides and matrix components with glass surfaces in the GC system. If standards in neat solutions are used for quantitation, which is the practice of federal regulatory labs, the accuracy of analytical results for affected pesticides in real samples is compromised. Other countries and many states avoid the matrix enhancement problem through the use of matrix-matched standards. In this study, a variety of SPE clean-up techniques after extraction of different foods with acetone or acetonitrile were evaluated in the attempt to eliminate the effect. Extensive clean-up did reduce the effect for certain pesticides, but the signal enhancement persisted for the more polar OPs.

DSI/GC/MS-MS+PFPD: Instrumentation to conduct studies using direct sample introduction (DSI)/GC/MS-MS + pulsed flame photometric detection (PFPD) in the analysis of pesticides in a variety of food and environmental matrices was obtained in September of 1998. DSI is a technique that permits the injection of samples in GC after a rapid extraction step without clean-up. A new DSI vial is used with every injection, thus, the approach is rugged because nonvolatile matrix components do not contaminate the column or MS source. The use of the very selective and sensitive PFPD and MS-MS overcomes background interferences, provides confirmation, and permits exceptionally low detection limits, particularly for OP insecticides. The approach has the potential to be the most rapid, inexpensive, efficient, and effective means to determine multiple pesticide residues in a variety of matrices yet devised.

IMPACT/TECH TRANSFER A: These studies impact the capabilities of regulatory and other laboratories to analyze pesticide residues in food. The use of lower cost, more rapid, easier, and higher quality methods can increase the monitoring rate, better ensure food safety, and provide more accurate data for risk assessment purposes. Also, more reliable data will lead to better science-based regulations and policies. The SFE pilot study and AOAC collaborative study have led a number of laboratories to begin using SFE and GC/MS for the

analysis of pesticide residues in foods. The NOAA Mussel Watch Project has requested the analysis of an additional 100 oyster samples collected from around the country using the SPE and GC/MS method for pesticide residues.

PUBLICATIONS:

Argauer, R.J., S.J. Lehotay and R.T. Brown. 1997. Determining lipophilic pyrethroids and chlorinated hydrocarbons in fortified ground beef using ion-trap mass spectrometry. J. Agric. Food Chem. 45:3936-3939.

Lehotay, S.J. 1997. Supercritical fluid extraction of pesticides in foods. J. Chromatog. 785:289-312.

Lehotay, S.J. 1998. New technologies applied in pesticide residue analysis. *In:* A. Valverde-García and A.R. Fernández-Alba (eds.), Pesticide Residues. IV Int. Sem. Pesticide Res., Institute Food Studies, Almería, Spain (in press).

Lehotay, S.J. 1998. Why are old methods hanging around? Inside Lab Management 2(6):35-39.

Lehotay, S.J. 1999. Strategies for multiclass, multiresidue analysis of pesticides. Encyclopedia Analytical Chemistry, John Wiley & Sons, New York.

Lehotay, S.J. and C.H. Lee. 1997. Evaluation of a fibrous cellulose drying agent in supercritical fluid extraction and pressurized liquid extraction of diverse pesticides. J. Chromatog. 785:313-327.

Lehotay, S.J., J.A. Harman-Fetcho and L.L. McConnell. 1998. Agricultural pesticide residues in oysters and water from two chesapeake bay tributaries. Mar. Poll. Bull. (in press).

Nemoto, S., and S.J. Lehotay. 1998. Analysis of multiple herbicides in soybeans using pressurized liquid extraction and capillary electrophoresis. J. Agric. Food Chem. 46:2190-2199.

DISPOSITION OF BETA-AGONISTS IN FARM ANIMALS

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OBJECTIVE A: Determine the metabolism, distribution, excretion, and elimination properties of beta-adrenergic agonists in food-producing animals.

PROGRESS A: A pharmacokinetic study of clenbuterol was conducted in 70 kg barrows (n=9). [^{14}C]Clenbuterol HCl (30 μg/kg bw) was delivered by intravenous (i.v.) administration followed by a 10 day depuration period, during which blood was collected from indwelling cephalo-brachial catheters. [^{14}C]clenbuterol was then orally administered to hogs at a similar dose, and blood samples were collected for a 10 day period. Plasma was assayed for total radioactive residues by liquid scintillation counting (LSC). Plasma clenbuterol was isolated using a validated solid-phase extraction procedure and concentration quantitated by LSC. Preliminary results indicate that total plasma radioactive residues (TRR) were best fit by the following models: $C_P = Ae^{-\alpha t} + Be^{-\beta t}$ (two-compartment system) or $C_P = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$ (three compartment system). After i.v. administration, plasma concentrations of TRR half-life values for the distribution (α), slower distribution (β), and terminal (γ) elimination phases had values of 0.013 $\pm$ 0.002, 12.2 $\pm$ 3.1, and 59.8 $\pm$ 21.7 hours (mean $\pm$ SEM), respectively. The apparent volume of distribution was 15.8 L/kg with an average clearance of 8.6 ml/min/kg. Preliminary data from the oral dosing experiments indicate that clenbuterol is rapidly absorbed in swine with peak plasma concentrations of radioactivity (9 ng/ml of plasma) occurring between 3 and 4 hours of administration. Direct comparison of the distribution and elimination of clenbuterol among target and non-target livestock species can now be made using these data.

IMPACT/TECH TRANSFER A: This study will provide basic data on the disposition of an illegally-used doping agent in a non-target species. Elimination characteristics of the drug can be compared directly to target species such as cattle and horses and generalizations regarding species differences, if present, may be inferred.

OBJECTIVE B: Determine the effects of β-adrenergic agonist (BAA) ractopamine (RAC), and its stereoisomers on muscle cells.

PROGRESS B: Ractopamine's mechanism of action *in vivo* could be through proliferation of satellite cells or hypertrophy of existing muscle cells. Muscle cell culture systems allow for evaluation of the direct effects of BAAs, removing the potential for extra muscular drug metabolism, and other indirect effects including fluxes in blood flow and nutrient concentration. While myoblasts serve as a model for satellite cells in growing muscle, myotubes are presumed to mimic the responses observed in differentiated muscle cells. A mouse muscle cell line derived from skeletal muscle was used (C_2C_{12}). Variables assessed were total DNA, total protein, cell number, and cyclic adenosine monophosphate (cAMP, a substance produced intracellularly and that acts as a second messenger). RAC (10μM) caused a ~30% increase in protein and DNA concentrations in myoblasts after 48h; no differences were found in myotubes. After maintaining the cell line in culture for several weeks, myoblasts failed to exhibit an increase in protein and DNA. Both myoblasts and myotubes increased cAMP production in response to 10μM RAC (4-7 fold control) in a 10 min incubation. RAC isomers ranked RR>>>SR>>RS>SS in ability to stimulate cAMP production, with essentially no response to SS, while SR produced about 50% of the RR response. β-adrenergic agonist propranolol (40μM) did not completely ablate RAC-stimulated cAMP production (~40% of RAC alone). In order to include an agriculturally-relevant model, cAMP response was also examined in turkey satellite cells (derived from *biceps femoris* of 12-week old Nicholas 88 toms) a species known to be responsive to ractopamine *in vivo*. While turkey satellite cells were responsive to forskolin (drug included as a positive control for cAMP stimulation), only RR of RAC produced even slightly elevated cAMP. The mouse system indicates RAC has a direct, acute (10 min) cellular effect on cAMP that can be assumed to be at least partially via β-adrenergic receptors in both myoblasts and myotubes. However, this effect does not correlate to a chronic effect (48h) on protein and DNA synthesis. Total DNA and protein in mouse myotubes was unchanged by RAC. Differences in stereoisomer activity are consistent with other BAAs, and indicate that stereo-specific metabolism is an important consideration in determining biological activity of a racemic mixture of RAC. There was no correlation between *in vivo* RAC effect and *in vitro* RAC effect on cAMP production. These results indicate RAC's effect on muscle cells may occur only partially through BAA receptors.

A rat study was conducted investigating the effects of ractopamine stereoisomers on growth and body characteristics of rats. Rats (150 g) intraperitoneally implanted with mini-osmotic pumps were studied for a 12 day period. Osmotic pumps contained saline, ractopmine (an equal mixture of 4 stereoisomers), RR, RS, SR, or SS stereoisomers of ractopamine. Growth rate, feed intake, urinary nitrogen, and fecal nitrogen were monitored during the study. Carcass (head, hide, bone, tail, muscle, heart, lungs, kidney, liver, and reproductive tract) and

viscera (gastrointestinal tract) were analyzed for chemical composition. Rats dosed with the RR stereoisomer responded similarly to rats dosed with ractopamine for all variables measured, with the exception of carcass lipid. Carcass lipid was decreased by ractopamine relative to controls, but was not different from controls in rats treated with the RR isomer. Compared to controls, body weight, carcass crude protein, and crude protein retention were increased by the RR stereoisomer and visceral lipid was decreased. In general, variables measured in rats dosed with the RS, SR, and SS isomers of ractopamine were not different from controls.

IMPACT/TECH TRANSFER B: These results indicate that the acute response of muscle cells does not necessarily reflect the chronic response. Therefore, further studies using mouse myoblasts will be performed to evaluate the action of each stereoisomer on total DNA and protein accumulation. In the whole animal, the RR isomer appears to mimic the effect of ractopamine (a mixture of 4 stereoisomers). Determination of the physiologically active stereoisomer of β-agonists will aid in determining the magnitude of risk associated with illegal residues of β-agonists in edible livestock tissues. Ractopamine has served as a model compound in this regard.

PUBLICATIONS:

Smith, D.J., G.D. Paulson and G.L. Larsen. 1998. Distribution of radiocarbon after intramammary, intrauterine, or ocular treatment of lactating cows with carbon-14 nitrofurazone. J. Dairy Sci. 81:979-988.

Smith, D.J. Total radioactive residues and clenbuterol residues in edible tissues, and the stereochemical composition of clenbuterol in livers of broilers after exposure to three levels of dietary [^{14}C]clenbuterol HCl and three pre-slaughter withdrawal periods. J. Anim. Sci. (in press).

ABSTRACTS:

Shappell, N.W., D.J. Smith, V.J. Feil, G.L. Larsen and D.C. McFarland. 1998. *In vitro* muscle cell response to ractopamine. J. Anim. Sci. 76 (suppl. 1):119.

DIOXINS IN BEEF, MILK, AND FORAGE

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OBJECTIVE A: Identify and quantify residues of chlorinated dioxins and furans in beef, chicken, and in animal feeds, in particular, forages. Devise strategies for minimizing the occurrence of these residues in order to minimize the impact on the consumer of meat products.

PROGRESS A: The United States Environmental Protection Agency (EPA) has designated beef as a major contributor to the human dioxin burden because animals grazing on forage contaminated by fallout from burning processes would store these lipophilic materials in their adipose tissue. Analyses of domestic beef samples in a study by FSIS/EPA and in our geographical survey (research facilities in thirteen states) generally showed low concentrations of dioxins and furans; however, some animals had concentrations that were many times greater than others. We have found that animals with high concentrations of dioxins were raised at facilities that contained pentachlorophenol (PCP) treated wood. Penn. State, Oregon State and Purdue Universities each had two production facilities. At each University we found treated wood components with high dioxin concentrations in the facility where the animals with high dioxin concentrations were raised; however, we found little or no evidence of PCP treated wood at each facility where animals with low dioxin burdens were raised (the production facilities were 5-10 miles apart).

One of our original goals was the determination of dioxin levels on forage that would have been deposited via airborne contamination. Our forage analyses have indicated that dioxins are present at levels below or near the limits of detection. Consumption of dioxins at these low levels should result in significant biomagnification during the feeding period of production animals, making dioxin analysis in animal fat a reliable measure of forage contamination. Because of PCP contamination at some of the production facilities, our data on this aspect have been limited. We theorized that since elk are not raised in confinement, dioxin concentrations in fat should be representative of area forage contamination. We have now analyzed kidney fat from elk taken from two areas in North Dakota and from an area in Wyoming near the

burn areas of Yellowstone National Park. Researchers have observed elk and other mammals foraging on the sugars in charred debris at the burn areas. Ironically the elk from the Yellowstone area were found to have very low dioxin levels while some of the elk from North Dakota were found to have moderately high levels. Collaborative efforts with personnel from the North Dakota Game and Fish Department have revealed feeding facilities in both elk ranges that were constructed for use during severe winter weather. Analyses of wood samples from these facilities revealed high dioxin concentrations. Other sources might be utility posts, fence posts and feeding facilities at farms near the elk ranges.

Studies with dairy cows and humans have shown that more hepta and octa dioxins often are excreted than consumed. Our studies have shown that PCP treated wood in feeding facilities is often associated with high concentrations of dioxins in beef fat. Fries and coworkers found that dairy cows fed PCP-treated wood excreted more dioxins than they consumed, further implicating PCP as a source of dioxins. Previously, our studies have shown that pure PCP is not a source of dioxins in rats, but that technical grade PCP is, suggesting that impurities in PCP contribute to the dioxin burden in animals and humans. We have now isolated a potential dioxin precursor (6-pentachlorophenoxy-2,3,4,5-tetrachlorophenol) from technical PCP and fed it to rats. Although only two percent of this "predioxin" was converted to octachlorodibenzodioxin, this amount may explain the discrepancies in mass balance studies that have been reported, provided that the predioxin is present in sufficiently high concentration. We are presently developing methodology to determine the concentration of the predioxin in treated wood.

IMPACT/TECH TRANSFER A: Elevated concentrations of dioxins in beef, when they occur, have invariably been traceable to pentachlorophenol treated wood in feeding facilities. Even elk, that are not confined, but have access to treated wood components, show somewhat elevated dioxin levels. These findings are contrary to the commonly accepted smoke stack to forage to animal route based on European data and provide information for modification of production procedures to suppress the impact of dioxins in humans.

OBJECTIVE B: Study the metabolic disposition and excretion of dioxins in animals so that it can be used to help minimize the occurrence of residues in mammalian tissues.

PROGRESS B: The absorption, disposition, metabolism, and excretion of a non-toxic dioxin congener (1,4,7,8-tetrachlorodibenzo-p-dioxin) (1,4,7,8-TCDD) was studied in a ruminating calf. Ninety four percent of a dose of ^{14}C-labeled 1,4,7,8-TCDD on grain was excreted from the calf within 96 h. Almost 60% of the dose appeared to not be absorbed and was isolated as parent compound from the feces. Metabolites isolated from the calf included hydroxylated tetra- and tri-chlorodioxins, which have also been found in rats dosed with 1,4,7,8-TCDD.

Unique to the calf was a dihydrodiol metabolite isolated from the feces. Dihydrodiols are formed through the action of epoxide hydrase enzymes on arene oxide intermediates. The isolation of a dihydrodiol metabolite further supports the idea of an arene oxide intermediate in the metabolism of dioxins. These reactive intermediates may be responsible for covalent binding to proteins observed for dioxin congeners, including congeners thought to be non-toxic. Although the majority of 1,4,7,8-TCDD was rapidly eliminated and metabolized in a ruminating calf, the formation of reactive arene oxide intermediates may result in covalent binding to biological macro-molecules.

A metabolism study of the toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) was conducted in conventional and bile-duct cannulated male Sprague-Dawley rats. Twelve rats were orally dosed with 2,3,7,8-TCDD at a rate of 1.25 mg/kg (CONV), while six bile-duct cannulated rats received an oral dose of 1.67 mg/kg (CANN). Urine, bile, and feces were collected at 24h intervals for three days. Feces was the major route of elimination (38.7% CONV and 81.0% CANN), although the vast majority of the fecal ^{14}C was present as the parent leading to the conclusion that the dose was not completely absorbed. Only 0.3% of the dose was present as metabolites in CONV feces. Metabolites identified were the NIH-shifted 2-hydroxy-1,3,7,8-TCDD, trichloromonohydroxy-CDD (a product of reductive dechlorination), and a tetrachlorodihydroxydiphenyl ether (a product of partial ring opening). Urine was a minor route of elimination (0.3% CONV and 0.6% CANN), and contained only metabolites. Bile was a minor route of elimination (0.5%), and also contained only metabolites. It was concluded that metabolism of TCDD was necessary for elimination via the urine or bile. Proteins present in the urine and bile served as binding species for TCDD metabolites. Albumin and α_{2u} were identified as targets for binding in the urine, while an uncharacterized 79kDa protein was the only species identified in the bile. The overall level of metabolism was extremely small, 0.5% per day. Liver was the major site of deposition (19.7% CONV and 4.5% CANN), and carcass, fat, and G.I. tract also contained significant levels. The liver-to-fat ratio was high, indicative of the induction of a liver protein binding species. Body weight losses were significant in both study groups (-11%) due to hypophagia.

IMPACT/TECH TRANSFER B: These studies with 1,4,7,8-TCDD describe the excretion, disposition, and metabolism of a non-toxic dioxin congener in a ruminating calf. The compound was rapidly excreted and extensively metabolized by the arene oxide pathway. The formation of a reactive arene oxide may be responsible for covalent binding to proteins observed for dioxins and may produce unwanted biological affects.

The studies with 2,3,7,8-TCDD show how slowly 2,3,7,8-TCDD is metabolized in rats. The metabolic pathway for toxic 2,3,7,8-TCDD is similar to that shown for the nontoxic 1,4,7,8-TCDD in that arene oxide intermediates are formed from which reductive dechlorination can occur. Retention of 2,3,7,8-TCDD residues in the liver is due the presence of a liver binding

species, which may prevent availability of 2,3,7,8-TCDD to the metabolizing enzymes. Prevention of liver sequestration of 2,3,7,8-TCDD may increase its metabolism and elimination, reducing its severe toxicity. Binding of metabolites to urinary and biliary proteins may alter their physiological function and represent a heretofore unidentified source of 2,3,7,8-TCDD toxicity. Lethal doses of 2,3,7,8-TCDD to the rat lead to significant body weight losses.

OBJECTIVE C: Develop alternative less expensive methods for the detection of dioxins in food.

PROGRESS C: Polyclonal antibodies generated from chickens were used to prepare immunoaffinity columns (IAC) that bound 1,3,7,8-TCDD and 2,3,7,8-TCDD in spiked serum or milk. The substrate was eluted by a non-ionic detergent, extracted, and applied to an acid silica column prior to GC-MS analysis. A column prepared with monoclonal antibody provided by Dr. Larry Stanker, ARS, Food Animal Protection Research Laboratory, College Station, TX, bound the TCDDs more tightly than the polyclonal antibodies. The TCDDs could be eluted by high concentrations of organic solvent (50% acetone). Serum samples needed to be diluted 1:20 to minimize the matrix effect in the polyclonal IAC, but no pre-column preparation was needed for the monoclonal IAC. Milk samples needed the same pre-column cleanup for both polyclonal and monoclonal IACs to ensure satisfactory results. Seventeen congeners of 13C-labeled and native TCDDs/TCDFs were spiked into serum for application to the monoclonal IAC. The congener binding pattern was analyzed by HRGC-MS, and the following congeners demonstrated satisfactory recovery (25-150%): 2,3,4,7,8-PeCDF; 2,3,4,6,7,8-HxCDF; 2,3,7,8-TCDD; 1,2,3,7,8-PeCDD; and 1,2,3,6,7,8-HxCDD. This demonstrated the monoclonal antibody column allowed the direct application of serum samples, and one solvent exchange gave appropriate samples for HRGC-MS analysis. The results showed immunoaffinity columns generated from monoclonal antibodies could provide individual congener data for TEQ calculations.

IMPACT/TECH TRANSFER C: Presently, available analysis of dioxins in food are too costly for regulatory monitoring. A more efficient sample cleanup could lead to simpler, faster analysis with a reduction in cost. Our studies indicate immunoaffinity columns may provide a route to providing more cost-effective monitoring of dioxins in food products. The switch from polyclonal antibodies to monoclonal antibodies will provide a continuous and uniform source for IAC generation.

PUBLICATIONS:

Feil, V.J., and G.L. Larsen. 1998. Dioxin and furan concentrations in elk lipids. Organohalogen Compounds 39:113-115.

Fries, G.F., V.J. Feil, R.G. Zaylskie, K.M. Bialek and C.P. Rice. 1998. Relationship of concentrations of pentachlorophenol and chlorinated dioxins and furans in wood from livestock facilities. Organohalogen Compounds 39:245-248.

Hakk, H., G. Larsen and V.J. Feil. 1998. Identification of a hydroxylated metabolite of 1278-TCDD in calf plasma following an oral dose. Organohalogen Compounds 37:389-393.

Hakk, H., G. Larsen and V.J. Feil. 1998. Tissue distribution, excretion, and metabolism of a high dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) in conventional and bile-duct cannulated rats. Organohalogen Compounds 37:351-355.

Huwe, J.K., and V.J. Feil. 1998. Major metabolites of 1,4,7,8-tetrachlorodibenzo-*p*-dioxin in a ruminating calf. Organohalogen Compounds 37:405-407.

Huwe, J.K., V.J. Feil., G.L. Larsen and C. Wiener. 1998. Metabolism and disposition of 1,4,7,8-tetrachlorodibenzo-*p*-dioxin in rats. Chemosphere (in press).

Huwe, J.K., V.J. Feil and T.O. Tiernan. 1998. *In vivo* formation of octachlorodibenzo-*p*-dioxin from a predioxin. Organohalogen Compounds 36:93-95.

Petroske, E., R.G. Zaylskie and V.J. Feil. 1998. Reduction of polychlorinated dibenzodioxin and dibenzofuran residues in hamburger meat during cooking. J. Agric. Food Chem. 46:3280-3284.

Shelver, W.L., G.L. Larsen and J.K. Huwe. 1998. The use of an immunoaffinity column for tetrachlorodibenzo-*p*-dioxin serum sample cleanup. J. Chromatog. B 705:261-268.

Shelver W.L., and J.K. Huwe. 1998. The application of immunoaffinity chromatography for the analysis of tetrachlorodibenzo-*p*-dioxin in bovine milk. J. Liquid Chromatog. Related Technol. (in press)

Shelver, W.L., and J.K. Huwe. 1998. Separation of PCDDs from biomatrices using an immunoaffinity column generated from a monoclonal antibody. Organohalogen Compounds 35:13-16.

APPLICATION OF SUPERCRITICAL FLUID TECHNIQUES TO FOOD SAFETY AND NUTRIENT ANALYSIS (Residue Analysis)

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OBJECTIVE A: Utilization of binary fluid mixtures for trace residue analysis.

PROGRESS A: Successful sample (extract) cleanup has been accomplished using binary mixtures of supercritical fluids at the appropriate pressure and temperature. Results have been reported in Analytical Chemistry as well as the subject of an application note of Scott Speciality Gases, Inc. Further negotiations with the above company regarding a CRADA arrangement were not successful, but the company does intend to market the subject fluid mixtures for regulatory analysis. We have constructed two additional fluid mixing modules in our laboratory for additional applications of this zero solvent cleanup method that eliminates more tedious chromatographic cleanup schemes. The associated flow controllers with these devices permit the use of carbon dioxide, propane, tetrafluoroethane (HFC-134a), and nitrogen in varying proportions. Internally, they are being applied for extract cleanup in our nutrient analysis program, process hydrogenation studies, and for use by outside collaborators. Some initial collaborative studies with FDA's Total Diet Research and Pesticide Center (TDRPC) in Lexena, KS (M. Hopper) have shown that combinations of SC-CO_2 and HFC-134a when applied to the cleanup of 24 spiked pesticides in butter fat, yielded above 75% recovery for 16 of the 24 pesticides while extracting only 6 mg of background fat. Similar promising results have been achieved on incurred residues in lamb chops and fish sticks.

IMPACT/TECH TRANSFER A: We are exploring other equipment and gas vendors to establish a commercialized outlet for this promising technique. A formal collaboration with FDA's TDRPC has been initiated; an additional transfer of this technology to other food monitoring laboratories will continue.

OBJECTIVE B: Derivatization reactions in supercritical carbon dioxide for carbamate pesticide determination.

PROGRESS B: Five derivatization agents were tested in the presence of SC-CO_2 to test their effectiveness for forming derivatives applicable for gas chromatographic analysis. These included acetyl chloride (to corrosive for SFE), chloroacetic anhydride (derivatization possible, GC/MS complex), HMDS/TMCS silane mixtures (sensitivity to moisture makes quantitative derivatization difficult) and hexafluorobutyric anhydride, HFBA, (high conversion rate, GC/ECD - No, but GC/MS analysis is possible). Additional studies focussed on optimizing the formation of the HFBA derivative, which for five model carbamate pesticides, gave conversions in excess of 98.5% at 8000 psi, 80°C, over a three hour reaction time (equivalent to that obtained in benzene as the derivatization solvent). Mass spectra of the derivatives were developed and used for quantitation. Detection limit by GC/MS (ion trap) was comparable to the FSIS-HPLC/fluorescent derivatization method; the HFBA derivatives were stable in n-hexane for at least six hours, and the method exhibited a reproducibility between 3-10% RSD with calibration curve linearity being exhibited between 50 ppb - 10 ppm. Using the most abundant ion peak, minimum detection limits between 0.48-5.4 ppb were achieved for the five carbamate pesticides. Interestingly, during the SFE-reaction sequence, increasing the CO_2 pressure decreased the reaction rate at 40 and 60°C, however at 80°C an increase in CO_2 pressure increased the reaction rate, which reduced the derivatization time to 1.5 hours. Due to the departure of the postdoctoral scientist working on the project (Z. Zhang), further work will be postponed pending the hire/arrival of a new scientist (permanent or postdoctoral hire).

IMPACT/TECH TRANSFER B: The above described procedure has the potential to provide an alternative method for analyzing carbamate pesticides of recent national concern. FSIS liaisons (Wilson and Soderberg) have interest in carbamate method to replace current FSIS Method CBM and this may offer an alternative if further developed and tested on food matrices.

OBJECTIVE C: Initial testing of a subcritical water extraction instrument for the determination of toxicants in food matrices.

PROGRESS C: A modified Spe-ed unit has undergone limited testing for the subcritical water extraction of pesticides. Spiked applesauce samples were subjected to subcritical water extraction at 200°C and 64 atmospheres and extraction of 2-4 ppm fortification of aldrin, dieldrin, heptachlor epoxide and endrin achieved. For the limited tests run, chlorinated pesticides can be recovered between 60-95% depending on the chosen extraction temperature and pressure. Calculations based on Regular Solution Theory indicate that a typical chlorinated pesticide can be solubilized in subcritical water at temperatures between 100-300°C at levels ranging from 0.1 to 100,000 ppm; in fact the solubility characteristics of the aqueous medium are very similar to a non-polar solvent at the quoted above quoted upper temperature limit (300°C). Further testing and method development has been curtailed resulting from a Category 1 scientist leaving, and pending negotiations with Applied Separations, Inc., a potential CRADA partner.

IMPACT/TECH TRANSFER C: Applied Separations, Inc now advertises a unit based on NCAUR research; and negotiations are continuing with the company to finalize a CRADA, including modification of the current unit to permit the destruction of small quantities of laboratory hazardous waste under supercritical water conditions. Because this extraction concept offers considerable promise and complements other "green" extraction technologies (i.e., SC-CO_2 extraction), a reimbursable research and development agreement may be signed with Dr. Hawthorne of the Energy & Environmental Research Center (Grand Forks, North Dakota) to expedite further subcritical water extraction studies.

PUBLICATIONS:

Hawthorne, S.B., and J.W. King. 1998. Principles and practice of analytical SFE, pp 219-282. *In:* M. Cause and D.Thiebaut (eds), Practical SFC and SFE. Harwood Publishers, Chur, Switzerland (in press).

Jarvenpaa, E.P., R. Huopalahti, J.W. King and S.L Taylor. 1998. Comparison of liquid modifiers used with supercritical carbon dioxide and their effect on analyte yield and the amount of interferences in the resultant extract. Proc. 5th Meet. Supercritical Fluids. Tome 2: Int. Soc. Adv. Supercritical Fluids, Nice, France, 693-698.

King, J.W., and Z. Zhang. 1998. Selective extraction of pesticides from lipid-containing matrices using supercritical binary gas mixtures. Anal. Chem. 70:1431-1436.

King, J.W. 1998. Analytical supercritical fluid techniques and methodology: Conceptualization and reduction to practice. J. Assoc. Off. Anal. Chem. Int. 8:9-17.

King, J.W. 1998. Integration of sample cleanup methods into analytical supercritical fluid extraction. Amer. Lab. 30(8):46-58.

King, J.W., and Z. Zhang. 1997. SFE starting to make headway in food analysis. Res. Devel. 39(11):CC-GG.

Snyder, J.M., J.W. King and Z. Zhang. 1998. Comparison of volatile analysis of lipid-containing and meat matrices by solid phase micro- and supercritical fluid extraction. *In:* C.J. Mussinan and M.J. Morello (eds), Challenges in Isolation and Characterization of Flavor Compounds, Amer. Chem. Soc., Washington, DC (in press)

Zhang, Z., and J.W. King. 1997. The effect of dissolved helium on the density and solvation power of supercritical carbon dioxide. J. Chromatogr. Sci. 35:770-773.

ABSTRACTS:

Taylor, S.L., J.W. King, J.I. Greer and J.L. Richard. 1998. Aflatoxin Elimination Workshop, J. Robens and J. Dorner (eds), p. 83.

Zhang, Z., and J.W. King. 1998. Derivatization reactions of carbamate pesticides in supercritical carbon dioxide. Abst. Pittcon '98, No. 414.

APPLICATION OF SUPERCRITICAL FLUID TECHNIQUES TO FOOD SAFETY AND NUTRIENT ANALYSIS (NUTRIENT ANALYSIS)

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OBJECTIVE A: Compare gravimetric and GC-FAME fat determinations of supercritical CO_2 extracts from a variety of foods.

PROGRESS A: SFE-based methods have been investigated to demonstrate the equivalence or non-equivalence of gravimetric and gas chromatographic (GC) fatty acid methyl ester (FAME) fat determination methods. Five oilseeds, three ground beef samples, five bakery samples and an NIST Standard Reference Material 1544 (SRM-1544) were extracted using supercritical CO_2 and (SC-CO_2)/ethanol. The collected material was weighed, and total fat determined gravimetrically (SFE-GRAV). Subsequently, an internal standard was added and the material converted to FAMEs and analyzed by GC to determine the SFE-GC-FAME total fat. The SFE-GC-FAME method was also compared to an acid hydrolysis/solvent extraction GC-FAME (AH-GC-FAME) method. The SFE-GRAV and SFE-GC-FAME fat determination methods were equivalent for 7 of 14 cases (i.e., soybeans, canola, safflower, 20 and 30% ground beef, cake with shortening, and cookies with emulsifier and shortening). For these cases, the SFE-GRAV method gives an accurate as well an easy and fast determination of total fat, and thus is an excellent alternative to solvent-based fat determinative methods for quality control situations. For the other 7 cases, the SFE-GRAV results were found to be significantly higher than the SFE-GC-FAME results. For moisture-laden samples, the observed difference is probably due to co-extracted water, and current studies are underway to bring the two methods into agreement by eliminating water from the extract before, during, or after extraction. The SFE-GC-FAME and AH-GC-FAME methods were equivalent in 7 of 9 cases, i.e., all three ground beef samples, cake with emulsifier, cake with shortening, cookies with emulsifier and shortening, and the SRM-1544. For these matrices, the SFE method is an excellent alternative to solvent-based methods for NLEA fat determinations. For the remaining two cases, the SFE-GC-FAME results were lower than the AH-GC-FAME results. This could be due to bound lipids not being extracted by the SFE method. This disagreement may be solved by the integration of a hydrolysis step into the SFE method.

IMPACT/TECH TRANSFER A: Using the above SFE total fat method, we assisted NIST in the analysis and certification of a Candidate Standard Reference Material 1546 "Meat Homogenate". Currently, similar studies are being conducted with the Leco Corporation on the use of SFE for the analysis of fat from bakery goods, including the use of starch digesting enzymes as a pretreatment step prior to sample extraction via SFE. Further testing of the above methodology will also be accomplished on the new HT-100 from Isco, Inc.

OBJECTIVE B: Investigate factors affecting gravimetric and GC-FAME fat determinations of supercritical CO_2 extracts from ground beef samples.

PROGRESS B: During earlier studies comparing gravimetric and GC-FAME fat determinations on supercritical fluid extracts of a 10% ground beef sample, it was noted that the SFE-GRAV results were significantly higher than the SFE-GC-FAME results and it was concluded that this difference was probably due to the coextraction of water with the fat. Consequently a study was initiated to investigate the effect of the ethanol modifier and sample drying (before extraction, during extraction and after collection) on fat determination for a 10% fat ground beef . The SFE-GRAV fat determination using CO_2/ethanol was significantly higher than the SFE-GRAV fat determination using neat CO_2, although the SFE-GC-FAME determinations for both methods were equivalent. Although drying the collected residue (in a vacuum oven for 30 minutes) after its collection, did decrease the SFE-GRAV fat determination for the CO_2/ethanol method, this result was still significantly higher than the subsequent GC-FAME fat determination, indicating that something other than water was being extracted. For ground beef samples dried before extraction and extracted with either neat CO_2 and CO_2/ethanol, the SFE-GRAV and SFE-GC-FAME determinations were significantly less than the corresponding determinations for undried samples. The GC-FAME determinations were equivalent for all extracts regardless of whether neat CO_2 or CO_2/ethanol was used as the solvent or whether the sample was extracted undried or dried after collection. In addition, several drying agents (i.e., anhydrous sodium sulfate, silica gel and 3Å molecular sieves) were evaluated for their ability to retain co-extracted water within the cell during the extraction. Both the SFE-GRAV and SFE-GC-FAME fat determinations using the 3Å molecular sieves, were significantly less than the fat determinations for all other methods. The SFE-GRAV and SFE-GC-FAME methods were equivalent for all the remaining treatments, (i.e., no drying agent, sodium sulfate and silica gel, or use of cosolvent, ethanol).

IMPACT/TECH TRANSFER B: These results will be further used to develop an SFE method for use in quality control analyses of meat products or in "Standard of Identity" analysis of ground beef products. Two commercial vendors of SFE equipment (Leco and Isco) have expressed interest in our laboratory participating in or running an AOAC Collaborative or Peer Verified study of the use of SFE for fat analysis of meat products.

OBJECTIVE C: Isolate, identify and determine the source of an artifact discovered during GC-FAME analyses of fat extracts of bakery products.

PROGRESS C: During studies comparing gravimetric and GC-FAME fat determination methods, it was noted that the GC-FAME fat determinations for acid-hydrolyzed bakery products were much higher than the expected level based on the ingredients in the product. The GC traces of these samples had a peak which did not correspond to any of the FAMEs in the standard mix. Mass spectral analysis of this peak gave a tentative identification as methyl 4-oxo-pentanoic acid (methyl levulinate). Proton and carbon nuclear magnetic resonance (NMR) spectroscopy supported this identification. Methyl levulinate was synthesized from levulinic acid and gave identical GC retention time, mass spectra and NMR spectra as did the original compound. Levulinic acid is a product of the acid hydrolysis of sugars in the bakery products and levulinic acid is methylated by the BF_3 used to trans-esterify the triglycerides. Although methyl levulinate could be detected in all bakery products containing sugar and hydrolyzed by hydrochloric acid, for high fat products, methyl levulinate constituted only a very small percentage of all the peaks in the GC trace, and its inclusion as a FAME had minimal effect on the calculated total fat. However, for low-fat/high sugar products, the methyl levulinate constitutes a large percentage of the total FAME peak areas, and if mistaken for a FAME, it can cause a significant overestimation of the actual fat content.

IMPACT/TECH TRANSFER C: The identification of this interfering compound was of considerable value to scientists at the American Institute of Baking (Manhattan, KS), who achieved agreement with fat levels based on predicted fat values determined from ingredient composition, by taking into account the erroneous response of the GC-FAME fat assay to the presence of levulinic acid in the sample matrix. The recognition of the presence of methyl levulinate in low-fat/high sugar products, which have undergone acid hydrolysis prior to solvent extraction, should lead to more accurate fat determinations for these types of foods, and the more precise assessment of fat levels in mixed meat/high carbohydrate matrices.

OBJECTIVE D: Analysis of sterols using analytical supercritical fluid techniques.

PROGRESS D: Fractionation of sterols from plant lipid mixtures was accomplished using a multi-step supercritical fluid extraction (SFE) procedure. Samples of seed oils, margarine, corn germ oil and corn fiber oil were extracted to yield extracts, enriched in phytosterol content. The analytical method employed an NH_2-bonded sorbent and utilized four discrete steps to separate and concentrate the sterols. Both SC-CO_2 and the use of SC-CO_2 with 10% organic modifier were required in this assay, which employed extraction pressures ranging

from 2000 - 8000 psi at 80°C. Capillary supercritical fluid chromatography (SFC) was also utilized to separate and determine the concentration of sterols in the extracts from the various samples. Enrichment of sterol concentration was ten-fold in many cases.

IMPACT/TECH TRANSFER D: The ability to separate and measure the amount of sterols in fat-containing foods is important to producers and consumers. concerned with the nutritional content of their products. This study indicates that it is feasible, using supercritical fluid-based extraction coupled with fractionation methods, to produce a substantial enrichment of sterols from seed oils. The method, which uses SC-CO_2 and a minimum of organic solvent, can be effective in analyzing the sterol (cholesterol) content of meats and other food matrices.

PUBLICATIONS:

Eller, F.J., and J.W. King. 1998. Supercritical CO_2 extraction of fat: Comparison of gravimetric and GC-FAME methods. J. Agric. Food Chem. (in press).

Eller, F.J. 1998. Interference by methyl levulinate in determination of total fat by GC-FAME analysis of low-fat/high sugar products. J. Assoc. Off. Anal. Chem. (submitted).

Frykman, H.B., J.M. Snyder and J.W. King. 1998. Screening catalytic lipase activities with an analytical supercritical fluid extractor. J. Am. Oil Chem. Soc. 75:517-520.

Snyder, J.M, J.W. King, S.L. Taylor and A.L. Neese. 1998. Concentration of phytosterols for analysis by supercritical fluid extraction. J. Am. Oil Chem. Soc. (submitted).

Taylor, S.L., F.J. Eller and J.W. King. 1997. A comparison of oil and fat content in oilseeds and ground beef using supercritical fluid extraction and related analytical techniques. Food Res. Int. 30:365-370.

POISONOUS PLANT TOXINS AND THEIR EFFECTS ON LIVESTOCK

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CRIS NUMBER: 5428-31320-002/5428-3200-008
CRIS TERMINATION DATE: July, 2002

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OBJECTIVE: Develop detection techniques and identify plant toxins in feeds, foods, and animal products or tissues. Determine the toxicokinetics (absorption, distribution, excretion and clearance) of plant toxins from the tissues or products of animals that consume poisonous plants.

The mission of the Poisonous Plant Research Laboratory is to identify toxic plants, isolate and identify plant toxins, determine the mechanism of toxicity, document toxin metabolism and clearance from tissues, develop diagnostic and prognostic procedures, identify conditions of poisoning, and develop management strategies, antidotes, treatments and other recommendations to reduce losses, insure product quality and promote animal and human health.

PROGRESS: Locoweeds (certain *Astragalus* and *Oxytropis* spp.) contain the toxin swainsonine. Swainsonine is rapidly absorbed: animals ingesting locoweed have this toxin in all tissue, including milk and other body secretions. Some tissues such as liver, pancreas, and kidney accumulate high swainsonine concentration (nearly 3000 ng/gm): this is nearly 10 times higher than serum concentration (generally 250 ng/gm). Clearance from the serum, skeletal muscle, heart and spleen is rapid (T ½˜20 hours), while swainsonine clearance (T ½) is slower at about 60 hours from the liver, kidney and pancreas. These results support recent findings suggesting that animals exposed to locoweed should be allowed a withdrawal time of about 28 days (10 Half lives) to insure swainsonine free animal products.

Pyrrolizidine alkaloid-containing plants are found throughout the world, and they often contaminate feeds and food. In a collaborative effort with the Natural Toxins Section of Australia's CSIRO, immunologic diagnostic techniques are being developed to quickly monitor feeds and food for possible contamination. Pyrrolizidine alkaloids form relative stable pyrrole adducts with tissue proteins and nucleic acids. Chemical techniques have been developed to detect these pyrroles in animal tissues. Little is known about the clearance, ultimate fate, or

toxicity of these adducts that could contaminate animal tissues. Current research objectives are to determine pyrrole kinetics, pyrrole toxicity, and the effects of low pyrrolizidine exposures on fetuses and neonates.

Some Lupine species contain quinolizidine and piperidine alkaloids that are toxic and teratogenic. Although these alkaloids have not been studied in animal tissues, they have been detected in the serum of poisoned animals. Additional studies are needed to better define the toxicokinetics and risks of these alkaloids.

Larkspur (*Delphinium* spp.) poisons thousands of animals yearly. Many more animals ingest less and do not appear to be clinically affected. These animals may also have larkspur alkaloids in many tissues. Initial work found that one alkaloid (methyllycaconitine) has a serum half-life of about 20 hours. Additional work is underway to better define larkspur alkaloid metabolism and excretion.

IMPACT/TECH TRANSFER: Research accomplished at the Poisonous Plant Research Laboratory has resulted in information, management strategies, diagnostic techniques and treatments for many poisonous plant problems. These results are important to the survival of rural ranching and small communities in many states. This work has increased producer and consumer knowledge of plant toxins, improved the technology to identify poisonous plant problems, improved animal productivity, and enhanced the utilization of pastures and rangelands where poisonous plants are found.

PUBLICATIONS:

Panter K.E., D.R. Gardner and R.J. Molyneux. 1998. Teratogenic and fetotoxic effects of tow piperidine alkaloid-containing Lupines (*L. formosus* and *L. arbustus*) in cows. J. Nat. Toxins 7:131-140.

Schoch R.K, D.R. Gardner and B.L. Stegelmeier 1999. GC/MS/MS detection of pyrrolic metabolites in animals poisoned with the pyrrolizidine alkaloid riddelliine. J. Nat. Toxins (submitted).

Stegelmeier B.L., L.F. James, K.E. Panter, et al. 1998. Tissue swainsonine clearance in sheep chronically poisoned with locoweed (*Oxytropis sericea*). J. Anim. Sci. 76:1140-1144.

Part V. NEW AREAS OF ARS RESEARCH

ANIMAL MANURE SAFETY

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CRIS NUMBER: 1270-12000-014/15
CRIS TERMINATION DATE: June, 1999

CRIS NUMBER: 1265-12320-001
CRIS TERMINATION DATE: April, 1999

OBJECTIVES: Careful use of manure was cited in the FDA draft "Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables" as a critical point in limiting potential contamination by pathogens that cause foodborne and waterborne illnesses. Field and operational practices for manure treatment and handling must avoid inadvertent introduction of pathogenic contaminants from fecal matter into growing and handling environments. Manure is often applied raw without treatment to stabilize nutrients or reduce odors or pathogens, when treatment processes like composting, drying, digestion, or lagooning are not practical. The BARC team planned and conducted studies to examine the growth, survival/destruction, and transport of pathogens in soil, treated manure, and fresh foods.

PROGRESS: *E. coli* O157:H7 strains grown in the lab and seeded into manure were shown to survive and actually replicate in soil during an 8 hr leaching experiment; *E. coli* readily leached through 4-inch packed cores of sandy loam soils. Experiments are in progress to determine the degree to which these strains move through other soil types and to test survival in the presence and absence of manures, plants, and plant residues. (J. Karns, Soil Microbial Systems Laboratory)

Methods were developed and optimized for the extraction and enumeration of *Cryptosporidium parvum* oocysts from feces/manures and soils (D. Shelton, Environmental Chemistry Laboratory). Experiments were conducted to identify the dominant factors controlling rate and extent of *Cryptosporidium parvum* oocyst movement through and/or infiltration into soils (D. Shelton, Environmental Chemistry Laboratory). These methods will be used for determining both the number and rate of oocysts filtered in both the laboraory scale soil boxes and field lysimeters.

Field lysimeters and a rainfall simulator were modified and constructed to determine relative rates and extent of *Cryptosporidium parvum* oocyst infiltration vs. runoff as a function of soil texture, slope, antecedent moisture content, and rainfall intensity/duration (D. Shelton, A. Sadeghi, A. Isensee, Environmental Chemistry Laboratory).

Plans were developed and agreements established with NVIRO International Inc. to evaluate the use of alkaline stabilization technologies for farm and larger scale treatment of manure to eliminate pathogens and odors, and stabilize nutrients in manure prior to land application in agricultural, forestry, and reclamation projects. Alkaline stabilization technologies include use of slaked lime, lime kiln dust, fly ash, concrete and other substances which can be mixed with manure to achieve a high pH and a high pulse of ammmonia to kill bacteria in the manure. Technology effectiveness for on-farm use will be compared with composting, digestion, drying, and lagooning at various scales of operation. Time-temperature criteria for pathogen reduction will be developed. (P. Millner, Soil Microbial Systems Laboratory)

Testing to determine pathogen kill in actual field/farm scale composting and alkaline stabilization processes. Laboratory composters are being upgraded so that work can commence early in FY99. (P. Millner, L. Sikora, Soil Microbial Systems Laboratory).

IMPACT/TECHNOLOGY TRANSFER: This research will be used by scientists in various agencies and sections within USDA (NRCS, ORACBA, FSIS, ARS), FDA, and USEPA (Office of Water, Office of Solid Waste) in their research on 1) detection and enumeration methods for pathogens from manure, 2) risk assessment, 3) on-farm manure management and treatment practices consistent with sustainable agriculture for various sizes of animal operations and integrated cropping systems, 4) detection, enumeration, and growth/die-off of pathogens on post-harvest crops in storage. The results will be used by NRCS and states in development of recommendations for manure management and by food processors and the fresh-cut industry in development of handling practices for fresh-cut produce that promote food safety and environmental health. As research progresses, results will be useful to develop guidance for 1) farmers relative to manure treatment, management, and land application, 2) food processors relative to handling and storage of fresh-cut and lightly processed produce, and 3) the water supply industry relative to transport (by leaching or runoff) of pathogen strains associated with agricultural vs. indigenous animal manures rather than human sources.

RAPID PATHOGEN DIAGNOSTIC AND DETECTION METHODS IN SHELLFISH

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G. Richards* CRIS TERMINATION DATE:

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OBJECTIVE A: To develop rapid detection and monitoring methods for pathogen and spoilage microorganisms in aquaculture process and products and to improve purification efficiency in order to prevent human illness.

PROGRESS A: A new worksite was opened at Delaware State University and building renovations to upgrade the site to a BL-2 facility were completed in August, 1998. Gary Richards was hired as the new Lead Scientist in March, 1998, and has overseen the renovations. The necessary equipment was purchased and installed to pursue research related to pathogens in aquaculture products, particularly molluscan shellfish.

IMPACT/TECH TRANSFER A: The manuscripts identify problems with enteric virus illnesses associated with molluscan shellfish and suggest actions to reduce the incidence of illness. The chapter on foodborne viruses for the Compendium of Methods for the Microbiological Examination of Foods transfers the latest technological advances in virus analytical procedures to regulatory agencies, academic institutions, and industry laboratories.

PUBLICATIONS:

Richards, G.P. 1998. Are viruses from shellfish a cause for concern? The Mid-Atlantic Aquafarmer. 23:4.

Richards, G.P. 1998. Limitations of molecular biological techniques for assessing the virological safety of foods. J. Food Protect. (submitted).

*** New SY Position**

INTERVENTIONS TO IMPROVE THE MICROBIOLOGICAL SAFETY AND QUALITY OF FRUITS AND VEGETABLES

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CRIS NUMBER: 1935-41420-003
CRIS TERMINATION DATE: September, 1999

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OBJECTIVE A: Develop means of decontaminating fresh produce containing human pathogens.

PROGRESS A: Intact, cut and punctured apples, inoculated with non-pathogenic surrogates of *Escherichia coli* O157:H7, were washed with various conventional and experimental wash formulations to determine their efficacy in decontaminating apples. Solutions containing 200 ppm chlorine, various acidic or alkaline detergent formulations, and 1000 ppm peracetic acid were similar in efficacy, reducing bacterial populations by only 1-2 logs. However, solutions containing 5% hydrogen peroxide, alone or in combination with acidic detergents and heated to 50C, achieved 3-4 log reductions in intact and cut apples. These treatments were substantially less effective with apples inoculated in punctures, and some evidence of *E. coli* growth within punctures was obtained. Field tests of promising decontamination treatments, carried out at the National Food Processors Association laboratory in Dublin, CA. in October, 1997 and June, 1998, provided information used to modify wash formulations and methods of treatment. Infiltration of *E. coli* O157:H7 into apple core tissue was demonstrated when Golden Delicious apples were immersed in a bacterial cell suspension, and the fruit temperature exceeded that of the water. A BL-2 pilot plant-scale fruit and vegetable washing and waxing system, provided with containment, remote operation and a decontamination system, was designed for decontamination experiments with commodities inoculated with human pathogens. Specifications for this system were prepared, and efforts are underway to identify potential vendors. A $420,000 grant was received from the Fund for Rural America for apple-related research, and recruitment of a research associate to perform this research as well as scientists to fill 2 SY positions are in progress. Decontamination treatments, found to be effective with inoculated apples, were applied to intact cantaloupes prior to preparation of fresh-cut melon cubes. Endogenous microflora were reduced significantly by treatment with hydrogen peroxide, and product shelf-life was extended significantly.

IMPACT/TECH TRANSFER A: The apple decontamination studies have demonstrated the risk of temperature-driven bacterial infiltration and have shown that conventional methods of washing are largely ineffective and that even experimental methods cannot achieve a 5-log reduction. These results were communicated to the industry and to other scientists at the State and Federal level through close interaction with the U.S. Apple Association; participation in workshops and meetings held in Ohio, Pennsylvania and Illinois; and presentation of a paper at the Institute of Food Technologists Annual Meeting. The investigators have been in close contact with FDA officials and scientific staff in planning collaborative research on sources of contamination and effective interventions for use at the FDA's Placerville, CA cider mill location.

OBJECTIVE B: Examine dynamics of interactions between spoilage microorganisms, foodborne human pathogens, and saprophytic epiphytes on fresh and minimally processed fruits and vegetables.

PROGRESS B: Factors affecting the survival and growth of foodborne human pathogen on fresh fruits and vegetables were investigated. Strains and species of foodborne human pathogens were examined for production of pectic enzymes that may be required for them to grow on plants. Display of pectolytic activity is common among *Yersinia* species or strains examined. The genes coding for two pectinases, identified as endo-pectate lyase and exo-polygalacturonase, have been cloned, sequenced and characterized.

The dynamics of interactions between soft-rotting bacteria and *Listeria monocytogenes* on fresh potato tuber slices were also investigated. Growth of *L. monocytogenes* on fresh potato tuber slices was almost completely inhibited in the presence of two species of soft-rotting fluorescent pseudomonads, but was not greatly affected in the presence of soft-rotting *Erwinia* or *Xanthomonas*. Results from a series of tests show that growth inhibition by pseudomonads is mainly due to the production of iron-chelating fluorescent siderophores (pyoverdin) by pseudomonads.

IMPACT/TECH TRANSFER B: Successful cloning of pectinase genes from *Y. enterocolitica* would now make it possible for us to construct an isogenic non-pectolytic strain to determine the role of pectinase production in the survival and growth of this human pathogen in plants. This knowledge could lead to the development of new strategies to prevent its growth in plants and fruits and vegetables. The exopolygalacturonase produced by *Yersinia* is unique in some properties and may be useful for modification of pectins for commercial application.

Results obtained from the ecological study also point to a possibility of using beneficial fluorescent pseudomonads as biocontrol agents to suppress the growth of foodborne pathogens such as *L. monocytogenes* on fresh and minimally processed fruits and vegetables. This study also shows that it is important not to re-contaminate fruits and vegetables with foodborne pathogens after disinfection treatments.

OBJECTIVE C: Develop means to improve the microbiological safety of sprouts.

PROGRESS C: Initial studies indicate that the use of competitive exclusion is a viable hurdle for the control of Salmonella on alfalfa sprouts. Of the 67 bacterial strains tested to date for their antagonism of *Salmonella*, several were found to give a 3 log reduction. One strain gave a 5 to 6 log reduction of *Salmonella* populations. Control of microbial populations on growing sprouts by addition of chemicals to the irrigation water was not effective. Using scanning electron microscopy, natural biofilms were demonstrated to be abundant on a variety of sprouts purchased from retail outlets. The presence of biofilms makes chemical control problematic.

IMPACT/TECH TRANSFER C: Research results were presented by invitation at a meeting of representatives of industry, academia and government at the University of Georgia-Griffin, February 16, and at the 9th Annual Meeting of the International Sprout Growers Association, San Diego, August 7 and 8. Results will also be presented by invitation at a public meeting on sprouts sponsored by the FDA Center for Food Safety and Applied Nutrition on September 28-29 in Washington, D.C.

PUBLICATIONS:

Buchanan, R.L., S.G. Edelson, R.L. Miller and G.M. Sapers. 1998. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. Food Microbiol. (submitted)

Janisiewicz, W.J., W.S. Conway, M. Brown, G.M. Sapers, P. Fratamico and R.L. Buchanan. 1998. Fate of *Escherichia coli* O157:H7 on fresh cut apple tissue and its transmission by fruit flies. Appl. Environ. Microbiol. (submitted)

Liao, C-H., and D.E. McCallus. 1998. Biochemical and genetic characterization of an extracellular protease from *Pseudomonas fluorescens* CY091. Appl. Environ. Microbiol. 64:914-921.

Liao, C-H., and G.M. Sapers. 1998. Influence of soft rot bacteria on the growth of *Listeria monocytogenes* on potato tuber slices. J. Food Protect. (submitted)

Liao, C-H., L, Revear, A. Hotchkiss and B. Savary. 1998. Cloning, sequence, and characterization of an exopolygalacturonase from *Yersinia enterocolitica*. Appl. Environ. Microbiol. (submitted)

Sapers, G.M., and G.F. Simmons. 1998. Hydrogen peroxide disinfection of minimally processed fruits and vegetables. Food Technol. 52(2):48-52.

Sapers, G.M., R.L. Miller and A.M. Mattrazzo. 1998. Efficacy of sanitizing agents in inactivating *Escherichia coli* in cider apples. J. Food Sci. (submitted)

ADHESION OF HUMAN PATHOGENS TO SURFACES OF FRUITS AND VEGETABLES

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CRIS NUMBER: 5325-41000-022
FSIS CODE: W1MC02
CRIS TERMINATION DATE: November, 1999

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OBJECTIVE A: To identify and describe the molecular mechanisms of the attachment of pathogens to surfaces of fruits and vegetables.

PROGRESS A: More than 50 strains of *E. coli* O157:H7 isolated from manure have been obtained and will be used for attachment studies and development of simpler strain differentiation methods. Multiple *E. coli* O157:H7 reporter strains have been prepared and will be used for studies of attachment/internalization to or in lettuce.

OBJECTIVE B: To identify new technology, including new compounds, that can minimize pathogens in foods.

PROGRESS B: Studies of attachment of *E. coli* O157:H7 to lettuce are ongoing. *E. coli* O157:H7 reporter strains have been produced for mirotiter well fluorescence and luminescence assays to detect attachment and potential attachment inhibitors. Plant extracts, essential oils and purified compounds have been screened for antimicrobial activity for pathogens. Some lead compounds have been identified.

IMPACT/TECH TRANSFER: Information on the attachment of pathogens in foods (e.g., single organism, biofilms, exterior/interior surface, etc.), and mechanism of pathogen binding to food surfaces will help producers develop and test strategies to control the amount of viable pathogen contamination and inhibitors of attachment, and/or compounds for controlling the amount of viable pathogen contamination.

PUBLICATIONS:

Kaper, J.B., L.J. Gansheroff, M.R. Wachtel and A.D. O'Brien. 1998. Intimin-mediated adherence of shiga toxin-producing *Escherichia coli* and attaching-and-effacing pathogens. p. 148-156, *In:* J.B. Kaper and A.D. O'Brien (ed.), *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* strains. ASM Press, Washington, DC.

PATENTS:

McKee, M., A. O'Brien, and M. Wachtel. Histidine-tagged intimin and methods of using intimin to stimulate an immune response and as an antigen carrier with targeting capability. United States and International Patent Applications filed.

Stewart, C. Jr., M. McKee, A O'Brien and M. Wachtel. Method of stimulating an immune response by administration of host organisms that express intimin alone or as a fusion protein with one or more other antigens. United States and International Patent Applications filed.

REDUCTION IN FUNGICIDE USE BY DETERMINING ALTERNATE STRATEGIES TO CONTROL POSTHARVEST DECAY

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OBJECTIVE: To determine the potential for foodborne pathogens to survive and multiply on fresh-cut apple fruit.

PROGRESS: Preliminary results indicate that the human foodborne pathogen *Listeria monocytogenes* can grow and multiply on the surfaces of apple slices at 20°C. This pathogen can survive at 5 and 10°C but multiplies very slowly. Controlled atmosphere storage conditions of 0.5 % O_2 and 5 % CO_2 or 0.5 % O_2 and 15 % CO_2 had little effect on controlling this microorganism. The pathogen, however, does not grow well on immature or freshly harvested fruit and seems to be a problem only on more mature or senescing fruit.

IMPACT/TECH TRANSFER: This work contributes to a basic understanding of the potential for apple slices to become contaminated with *Listeria monocytogenes* and provides information for the fresh produce industry showing that the problem may be especially important in fruit stored for longer periods of time. At this time, controlled atmosphere storage does not seem to offer a viable method of controlling *Listeria monocytogenes*, so the fresh-cut industry should consider other methods of control.

ADVANCED TECHNOLOGIES FOR REDUCTION OF MICROORGANISMS AND PARTICULATE MATTER IN FOOD PROCESSING

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CRIS NUMBER: 5325-42000-024
FSIS CODE: W2CC01
CRIS TERMINATION DATE: July, 2002

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OBJECTIVE: Reduce the need for excess chlorine or chlorine dioxide by developing new methods for sterilization or pasteurization of food.

PROGRESS: Chlorine dioxide (acidified chlorate) was established in accelerated storage tests to be an effective disinfectant for control of bacterial soft-rot in potatoes. The disinfection process was also expected to prevent storage spoilage of potatoes infected by late blight fungus. This research was done in collaboration with BCI under a CRADA.

IMPACT/TECH TRANSFER: The State of Idaho petitioned USEPA to grant a permit for immediate test of the process in Idaho and Washington because of the wide spread of late blight infection in the Northwest. A permit was issued to Idaho to test a maximum of 2,000,000 tons of stored potatoes. A separate permit was issued to the State of Washington to treat 2,500,000 tons. A field test is being organized with the University of Idaho, the Idaho Department of Agriculture and the potato industry in Idaho.

PUBLICATIONS:

Tsai, L-S., C.C. Huxsoll and B.T. Molyneux. 1998. Prevention of potato spoilage during storage by chlorine dioxide. J. Food Protect. (submitted)

OPTIMIZATION OF SAFETY, QUALITY, AND SHELF-LIFE ON SPROUTS AND SEEDS

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CRIS NUMBER: 1935-42000-033
FSIS CODE: E2M102
CRIS TERMINATION DATE: April, 1999

OBJECTIVE A: Determine if *E. coli* O157:H7 and *Salmonella* on sprouts and seeds can be inactivated by treatments with gamma radiation while maintaining product quality.

PROGRESS A: Responding to a request from FDA we have initiated an investigation of the effects of gamma radiation on sprouts, the sproutability of seed, and the radiation resistance of *E. coli* O157:H7 and *salmonella* on these products. Initial results indicate that the study should be pursued to determine if this is a viable technology for the elimination of foodborne human pathogens from both seed and sprouts.

IMPACT/TECH TRANSFER A: Unknown; however, there have been outbreaks of disease linked to alfalfa sprouts in the United States, and there is considerable concern and effort to develop a suitable treatment for seed and sprouts.

OBJECTIVE B: Evaluate the ability of ionizing radiation to inactivate *Cyclospora cayetanensis*.

PROGRESS B: The effect of ^{137}Cs irradiation on unsporulated and sporulated *Toxoplasma gondii* oocysts was investigated as a model system for sterilisation of fruit contaminated with other coccidia such as *Cyclospora* or *Cryptosporidium*. Unsporulated oocysts irradiated at ≥ 0.4 to 0.8 kGy sporulated but were not infective to mice. Sporulated oocysts irradiated at ≥ 0.4 kGy were able to excyst, and sporozoites were infective but not capable of inducing a viable infection in mice. *Toxoplasma gondii* was detected in histologic sections of mice up to 5 days but not at 7 days after feeding oocysts irradiated at 0.5 kGy. Transmission electron microscopy revealed that sporozoites from irradiated oocysts penetrated eterocytes and all cells in the lamina propria except for red blood cells. Sporozoites appeared normal ultrastructurally and formed a typical parasitophorous vacuole containing a well-developed tubulovesicular membrane network.

Raspberries inoculated with sporulated *T. gondii* oocysts were rendered innocuous after irradiation at 0.4 kGy. Results indicate that irradiation at 0.5 kGy is effective in "killing" coccidian oocysts on fruits and vegetables.

IMPACT/TECH TRANSFER B: These results have been communicated to the FDA.

IMPROVING RUMINAL FERMENTATION

ARS Contact Persons:
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OBJECTIVE: Determine the effect of cattle diet on the acid-resistance of *Escherichia coli*.

PROGRESS: Most food-borne pathogens are killed by the low pH of gastric fluids, but native strains of *E. coli* can sometimes survive acid shock. It had long been recognized that *E. coli* develops extreme acid resistance when it is grown under mildly acidic conditions, but the inducer of this trait had not been identified. Experiments with *E. coli* O157:H7 indicated that undissociated volatile fatty acids were the inducer. If the total volatile fatty acid concentration was high, there was enough undissociated volatile fatty acid to induce extreme acid resistance even if pH was near neutral. When cattle were fed large amounts of grain (> 45% of DM), volatile fatty acids accumulated in the colons of cattle, and pH declined. Grain-feeding also cause a 1,000-fold increase in total *E. coli* numbers, and a 1,000-fold increase in acid resistance. Based on numbers and survival after acid shock, cattle fed grain had 1,000,000-fold more acid-resistant *E. coli* than cattle fed hay. When cattle we switched from grain to hay, *E. coli* numbers and the acid-resistant count decreased. After 5 days acid-resistant *E. coli* could no longer be detected.

IMPACT/TECH TRANSFER: This work was cited or discussed by a wide range of Journals, Newspapers: including Science, New York Times and the Washington Post; national public radio and various television stations.

PUBLICATIONS:

Diez-Gonzalez, F., T.R. Callaway, M.G. Kizoulis and J.B. Russell. 1998. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. Science 281:666.

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